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The Impacts of Agricultural Land Management on Soil Carbon Stabilisation

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Declaration

I confirm that the work submitted within this thesis is my own, except where work was carried out in collaboration with others. The contribution of others is explicitly acknowledged below. Appropriate credit is given within the thesis where reference is made to the work of others.

The modelled results presented in Chapter 4 were produced by Dr Sarah Buckingham. Fractionation work presented in Chapter 5 was conducted in collaboration with Dan Chen and part of the data was submitted in fulfilment of her MSc dissertation. I confirm that I undertook a substantial amount of the work presented in that chapter.

Gemma Miller

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Abstract

Soil is the largest terrestrial carbon (C) store, containing an estimated ~1500 Gt C in the upper 1 m of soil. The long term storage of soil organic C (SOC) requires that it is somehow protected from microbial decomposition – or ‘stabilised’ – in the soil matrix. Three mechanisms are commonly identified as factors controlling the stability of SOM: chemical recalcitrance, physical protection in aggregates and adsorption to soil mineral surfaces. The stability of SOC in the soil matrix can be influenced by management practices and changes in soil structure can lead to loss of SOC and increases in greenhouse gas (GHG) emissions. It is, therefore, important to understand the impact that management practices have on SOC stability and to manage soils in such a way as to optimise the volume of SOC which is locked away for climatically significant periods of time.

Two methods are generally used to estimate SOC stability: indirectly by measuring CO₂ fluxes as a proxy for SOC microbial decomposition, or directly through physical fractionation of soil into pools with different levels of physical and chemical protection. Both methods were employed in this thesis.

Arable and grassland soils which represent the range of soil textures and climatic conditions of the main agricultural areas in the UK were incubated at two different moisture contents and with or without inorganic fertiliser application and GHG fluxes from them were monitored. Soil texture, mineral N concentration and soil C concentration were found to be the most important measured variables controlling GHG fluxes of the UK agricultural soils in this study. The results were generally in support of those found in the literature for a wide range of soils, conditions and locations; however, N₂O emissions from the two Scottish soils appeared to be more sensitive to inorganic N fertilisation at the higher moisture content than the other soils, with the N₂O emissions being exceptionally high in comparison.

Although incubations of whole soils are useful in measuring the impacts of soil management practices on GHG emissions under controlled conditions they do not identify the mechanisms controlling the stability of SOC.

Dividing SOM into functional pools may identify different C stabilising mechanisms and improves soil C models. A large number of operationally defined separation methods have been used to fractionate SOM into biologically meaningful pools of different stability. Direct comparisons of different fractionation methods using radiocarbon (¹⁴C) dating and spectroscopic analyses has not previously been undertaken. Average ¹⁴C ages and chemical composition of SOM fractions isolated from a grassland soil using three published and

frequently applied fractionation methods were compared. (1) a density separation technique isolating three fractions (2) a combined physical and chemical separation isolating five fractions (3) a hot-water extraction method isolating two fractions. The fractions from Method 1 had the most distinct average ^{14}C ages, the fractions from Method 2 fell into two age groups, and both Method 3 fractions were dominated by modern C. The average ^{14}C ages of the labile fractions from Method 1 and 2 were higher than the mineral bound fractions, although they made up a relatively small proportion of the total SOC. This was a surprising result, and spectroscopic analysis confirmed that these fractions had greater relative contents of aliphatic and aromatic characteristics than the mineral bound fractions. The presence of black C in a whole soil sample and one of the labile fractions from Method 2 was confirmed by hydrogen pyrolysis.

The availability of archived soils from an abandoned long term tillage treatment experiment and the ability to relocate the plots provided a unique opportunity to assess the resilience of SOC stocks to land management practices several years after the conversion from arable to grassland. SOC stability was assessed by soil fractionation of archived (1975) and freshly collected (2014) soil samples. The mass corrected SOC stocks from the four different treatments (deep plough, shallow plough, chisel plough and direct drill) were higher in 2014 than 1975 across the whole profile (0 – 36 cm). Reductions were observed at some depths for some treatments but the overall effect was an evening out of SOC stocks across all plots. The fractionations (using Method 2), revealed that there was a relative increase in the mass of the sand and aggregate fraction but a decrease in the relative proportion of SOC stored in this fraction (physically protected). There was also a significant increase in the C:N ratio of the silt and clay fraction (chemical adsorption). This suggests that reduced disturbance of agricultural soils leads to preferential physical stabilisation of fresh SOM but also increased adsorption of older material to mineral surfaces. The labile fractions were sensitive to land-use change in all tillage treatment plots, but were more sensitive in the low impact tillage plots (chisel plough and direct drill) than the inversion tillage plots (deep plough and shallow plough).

It is well established that tillage disrupts aggregation. However, a direct measurement of the level of SOM physical protection in the soil matrix due to aggregation has not previously been undertaken. The soil was fractionated using Method 1 (fractions with distinctly different ^{14}C ages) and isolated soil fractions were incubated separately, recombined and mixed in to whole soil at three different temperatures. The C respiration rate of the isolated intra-aggregate fraction was generally consistently as high as the whole soil. This supports

the theory that there is a labile component of soil which is protected from decomposition by physical protection within aggregates. Therefore, the lack of any priming effect with the addition of labile fractions to the whole soil, and indeed the suppression of emissions relative to the whole soil, was unusual. Fractions and whole soils incubated at 25 and 35 °C had a wider range of Q_{10} (temperature sensitivity) values than those incubated at 15 and 25 °C, however, median values were surprisingly similar (range from 0.7 to 1.9).

Overall, the results from this thesis highlight the importance of the soil structure in stabilising C. Disrupting aggregates leaves a proportion of otherwise stable C susceptible to loss through microbial decomposition, particularly when the entire soil matrix is disrupted. It also provided some unexpected results which warrant future investigation; in particular, further direct measurement of physical stabilisation of SOM in soils of different type, from different climates and different land uses would be useful.

Lay Summary

Soil contains vast quantities of carbon (C) and can keep it locked away from the atmosphere for hundreds to thousands of years. The long term storage of soil organic C (SOC) requires that it is somehow protected from microbial decomposition – or ‘stabilised’ – in the soil matrix. Three mechanisms are commonly identified as factors controlling the stability of SOM – inherent resistance to decomposition, physical protection in aggregates and chemical bonding to soil mineral surfaces. The stability of SOC in the soil matrix can be influenced by management practices and changes in soil structure can lead to loss of SOC and increases in greenhouse gas (GHG) emissions. It is, therefore, important to understand the impact that agricultural management practices have on SOC stability and to manage soils in such a way as to optimise the amount of SOC which is locked away for climatically significant periods of time. The aim of this research was to investigate the effects of specific agricultural land management practices on SOC stability.

This aim was achieved through a series of laboratory experiments which either indirectly measured SOC stability by assessing the amount of carbon dioxide (CO₂) respired from the soil (as a proxy for SOC decomposition), or directly by physically breaking it down into ‘pools’ which perform different functions and characterising their chemical properties. These experiments investigated the factors controlling CO₂ (and other GHG) emissions after inorganic nitrogen fertiliser application to a range of UK arable and grassland soils under different moisture conditions, tested the resilience of SOC after land-use change from arable to grassland and directly measured the importance of soil structure in maintaining SOC stability.

Overall, the results from these experiments highlighted the importance of physical soil structure in stabilising SOC. Disrupting aggregates leaves a proportion of otherwise stable C susceptible to loss through microbial decomposition, and although aggregation is improved after cessation of tillage, increased SOC protection lags behind. This research also provided some surprising results which warrant future investigation. In particular, further direct measurement of physical protection of SOM in different types of soils, from contrasting climates and different land uses would be useful.

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1 Introduction

The global carbon (C) cycle is a dynamic exchange of C between the atmosphere, the oceans and the terrestrial environment which is driven by both natural and anthropogenic processes. The oceanic and terrestrial pools (vegetation, soil and detritus) are both substantial reservoirs of C (Ciais *et al*, 2013, Figure 1.1) but the terrestrial pool receives particular attention due to its accessibility and intensive exploitation by humans. Globally, soil contains a significant C stock with an estimated ~1500 Gt C down to 1 m (excluding permafrost soils, Batjes, 1996), which is around twice that in the atmosphere (800 Gt C) and three times that stored in vegetation (420-620 Gt C) (Ciais *et al*, 2013; Powlson *et al*, 2011).

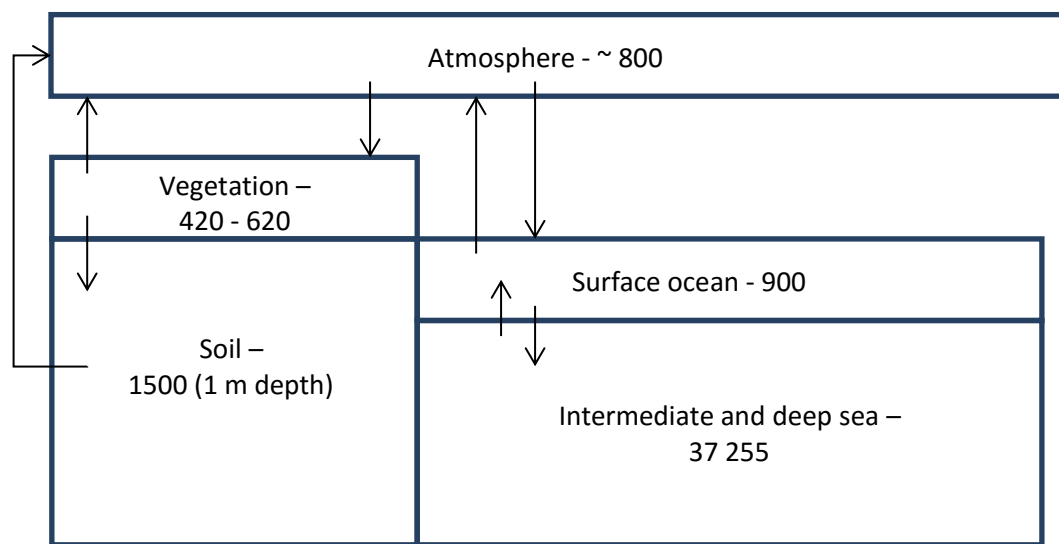


Figure 1.1: Estimated global C stocks (Gt C) in terrestrial, oceanic and atmospheric pools. Arrows show flows between pools. Simplified diagram from Ciais *et al* (2013).

The importance of soil as a sink for atmospheric C to mitigate climate change has received much attention in the literature in recent years, although it has been noted that earlier estimates of the potential increase in soil organic C (SOC) sequestration may have been overstated (Powlson *et al*, 2011). Regardless of this, it is important that soils are managed in such a way as to optimise the volume of SOC retained in the soil for climatically significant periods of time (decades to centuries).

Long term storage of SOC requires that the C is somehow protected from microbial decomposition – or ‘stabilised’ – in the soil matrix. Three mechanisms are commonly identified as factors controlling the reactivity of soil organic matter (SOM) - inherent chemical recalcitrance, physical protection in aggregates and adsorption to soil mineral surfaces (Six *et al*, 2000; Krull *et al*, 2003; von Lützow *et al*, 2006). The difference in physical location of these different SOM pools, the varying ability of microbes to utilise them and their different functional roles lead to differences in their stability within the soil

matrix, and so their dynamics (Golchin *et al*, 1994; Six *et al*, 1998). Environmental factors such as temperature, pH and moisture level are also contributory factors in the stabilisation of SOM (Krull *et al*, 2003; D'Angelo *et al*, 2009).

Land management practices, such as tillage, fertilisation, and land-use change can alter the stability of SOC by disrupting or improving the soil structure and/or influencing microbial activity. This can result in either losses of, or increased sequestration of, C in the soil, however, it is also important to consider the impact of management on greenhouse gas (GHG) emissions as these may counteract some of the benefit gained by increased C sequestration (Smith *et al*, 2008; Powlson *et al*, 2011).

1.1 Soil organic matter stabilisation mechanisms

1.1.1 Chemical recalcitrance of SOM

The timeworn theory that the fate of organic material entering soil is determined only by its chemical structure has been challenged in recent decades. Traditionally it was thought that labile chemical components (e.g. carbohydrates and proteins) were readily utilised by soil microbes and thus preferentially decomposed, followed by abiotic “spontaneous heteropoly condensation” reactions which form humic substances (von Lützow *et al*, 2006). Humic substances were deemed resistant to decomposition (Schmidt *et al*, 2011) and operationally defined by their solubility in acids and bases (Picollo, 2001; Dungait *et al*, 2012). Humic substances range widely in chemical characteristics and are commonly considered to be heterogeneous mixtures of chemical components (Stevenson, 1982; Picollo, 2001).

Some organic compounds such as alkyl C chains in lipids, aromatic structures in aromatics and phenolics are considered to be inherently recalcitrant (Lorenz *et al*, 2007). However, in a review Marschner *et al* (2008) found that most presumed inherently recalcitrant materials had high turnover rates or were readily decomposed on the addition of a labile substrate (inducing a priming effect), so challenging the existence of inherently recalcitrant SOM. Fontaine *et al* (2007) found that addition of fresh C to subsoil ‘reactivated’ decomposition of recalcitrant OM.

The emerging story of SOM has proved to be much more complex and has caused a paradigm shift away from terms associated with humic chemistry (von Lützow *et al*, 2007; Schmidt *et al*, 2011). Other theories have been developed to explain the persistence of unprotected SOM in soil, often referred to as free or particulate organic matter (POM) and normally isolated from soil by density separation (discussed in Chapter 2), which may work alone or in conjunction, and are described below.

Dungait *et al* (2012) postulate that, owing to the vast diversity of the microbial communities in soil and the enzymes which they produce, almost any organic matter has the potential to be decomposed under the right circumstances. Ekschmitt *et al* (2005) provide a list of common recalcitrant compounds found in SOM along with the sources of enzymes within soil which can decompose them. Recalcitrant SOM is generally considered to consist of complex, highly condensed aromatic, high molecular weight macro-molecules; however, the molecular structure may only influence the relative rate of decomposition (Krull, *et al*, 2003) with the “complexity of the decomposition operation” being the controlling factor (Kleber, 2010).

Kleber (2010) plotted respiration rate constants of various compounds from Fierer *et al* (2005) against some generally accepted molecular characteristics of recalcitrant material and found that there was no strong correlation between these properties and the decomposition rate of the compounds. However, they found that the availability of oxygen to the decomposer community, either atmospheric or bonded in substrate compounds, was much more important. Margenot *et al* (2015) found a lower ratio of aromatic to aliphatic functional group concentration as SOC concentration increased ($R^2 = 0.55$) and Kleber *et al* (2011) found lower O-alkyl C/aromatic C ratios in younger ^{14}C dated density fractions than in older fractions (see Fractionation methods Section 2).

A theoretical model based on an inverse of the Michaelis–Menten equation (Schimel and Weintraub, 2003) demonstrates that decomposition of SOM may be limited by the efficiency of the enzymes. As enzyme concentration increases in the surrounding soil matrix, the return to the organism in terms of resources and energy decreases until a ‘compensation point’ is reached, where it is no longer energetically viable for the organism to continue producing enzyme (Schimel and Weintraub, 2003; Ekschmitt *et al*, 2005).

There is some evidence to show that inherent chemical recalcitrance does play a role in SOC stabilisation. Mikutta *et al* (2006) hydrolysed twelve acid subsoils of varying mineralogy with NaOCl to yield a resistant and mineral-bound fraction, followed by dissolution of minerals with HF acid leaving only the resistant C. The resistant OC from the twelve subsoils had remarkably similar chemical structure (CPMAS ^{13}C -NMR spectra), with the predominant compounds being aliphatic in nature (specifically alkyl-C). This is, however, also consistent with the theory that oxygen availability is the key driver of recalcitrance as aliphatic compounds are oxygen depleted and the subsoils are isolated from atmospheric diffusion of O_2 (Kleber, 2010).

1.1.2 Occlusion within aggregates

Soil OM which becomes physically occluded within macro- or micro-aggregates are afforded protection from decomposition through the limiting of three processes by the

structure (pore size distribution and connectivity) of the aggregates: preventing access to SOM by microbes, reducing diffusion of microbial enzymes in to aggregates and oxygen depletion in pores through limited O₂ diffusion (von Lützow *et al*, 2006).

The formation of macro- and micro-aggregates is described by evidence referenced in Six *et al* (2000), it is summarised briefly here. When OM is added to soil (in the form of crop or plant residues, root exudates, dead roots, faeces, etc.) it becomes a substrate for microbial activity and is decomposed. During decomposition, microbes produce secretions which, along with fungal hyphae and plant roots, bind POM and soil minerals together into macro-aggregates (250–2000 µm in size). Macro-aggregates are transient by nature as the agents binding them together are degradable themselves. However, the duration of their existence is affected by land management practices which disturb the soil structure (discussed below).

The POM in macro-aggregates is, through time, broken down in to smaller pieces by microbial decomposition. During this process, POM (and microbially derived organic substances) becomes encrusted in clay particles and form highly stable micro-aggregates i.e. there is a change in the stabilisation mechanism from biotic to abiotic. Macro-aggregates eventually destabilise and micro-aggregates are released, becoming the building blocks for the formation of new macro-aggregates.

The classical theory of micro-aggregate formation is based on the assumption that minerals bind around OM (Tisdall and Oades, 1982; Golchin *et al*, 1994). However, Lehmann *et al* (2007) examined the structure of stable micro-aggregates and found that the SOM was distributed randomly throughout individual aggregates in discrete pockets which were isolated from microbes by minerals, or within small pores which microbes could not access. Organic matter enclosed in the core of aggregates also has a higher C:N than that on the outside (Amelung and Zech, 1996). Together, this suggests that OM is initially stabilised by adsorption on to mineral surfaces, eventually becoming encrusted in mineral particles, rather than first being occluded by minerals before bonding chemically.

Intra-aggregate OM is often assumed to be more highly decomposed (loss of recognisable structure and increase in aliphaticity), smaller in size and have longer mean residence times (MRTs) than the un-occluded (free) OM, although there is wide variation in the chemical characteristics of intra-aggregate OM in the literature (Wagai *et al*, 2009; Schrumpf *et al*, 2013).

There are no clear explanations for the variability of the SOM occluded in aggregates, although it may be due to the quality of SOM inputs and land management practices. Wagai *et al* (2009) propose that the intra-aggregate SOM is a mixture of partially

decomposed labile OM and recalcitrant material which is less decomposed and so has retained more of its original chemical structure. However, the relative importance of the physical occlusion and chemical recalcitrance in increasing the MRT of intra-aggregate material is unknown (Schrumpf *et al*, 2013). Black C, which is highly stable, has also often been observed to congregate in the intra-aggregate SOM fraction (Brodowski *et al*, 2006).

1.1.3 Adsorption to mineral surfaces

Physico-chemical stabilisation of OM by adsorption to mineral surfaces (typically clay minerals and Al and Fe oxy/hydroxides) is well understood and the mechanisms have been described by Sollins *et al* (1996), Baldock and Skjemstad (2000) and von Lützow *et al* (2006). Clay particles are the most significant for OM adsorption as they have a high specific surface area and high charge density (Ransom *et al*, 1998; Baldock and Skjemstad, 2000) on which to interact with SOM. Soil texture is positively correlated with the amount of SOC in the silt and clay fraction of soil (Hassink, 1997; Six *et al*, 2002), this relationship varies for land management type and the dominant soil minerals (Six *et al*, 2002). It is possible for the clay fraction of soil to greatly increase the mass of the SOC it contains, without greatly increasing its volume.

Mechanisms of SOM adsorption onto mineral surfaces include ligand exchange (the exchange of OH groups between mineral surfaces and carboxylic or phenolic organic OH groups, or exchange of mineral and organic cations), polyvalent cation bridges (neutralisation of negatively charged clay surfaces and organic functional groups by Ca^{2+} , Fe^{3+} and Al^{3+}), and weak interactions such as hydrophobicity, Van der Waals forces and H-bonding (von Lützow *et al*, 2006). Ligand exchange has been found to create the strongest bond between clay minerals and SOM, followed by cation bridging (Mikutta *et al*, 2007). However, the relative contribution of each mineral stabilisation type, in terms of both quantity and stability of SOM adsorbed, is dependent on the mineralogy of the soil (Mikutta *et al*, 2007; Sanderman *et al*, 2014).

It is unclear as to how OM is protected from decomposition by adsorption to mineral surfaces. OM may be made inaccessible to microbes by occlusion within the structure of layered minerals (intercalation). However, microbial biomass and extra-cellular enzymes also have an affinity for adsorption on soil minerals which may effectively prevent either from coming in to contact with SOM (Dungait *et al*, 2012). Desorption of small molecules may have to occur before they can be utilised by micro-organisms (Chenu and Stotzky, 2002). Mikutta *et al* (2007) found that the easily desorbed, weakly bound SOM accounted for the majority of decomposed SOM in incubations of mineral-organic mixtures. The complexation of SOM with minerals may also increase the activation energy required for decomposition (Kaiser *et al*, 2002), thus slowing its rate.

Adsorption of larger organic molecules (such as proteins) causes changes to the conformation of the molecule (denaturation) which render extra-cellular enzymes incompatible (Quiquampoix, 2000).

1.2 Effects of land management practices on soil organic matter stabilisation

1.2.1 Tillage

Much of the blame for the loss of C from agricultural soils has been attributed to tillage, in light of which, no till (NT) and reduced till systems have been adopted as alternatives to conventional till (CT) in many agricultural systems as a means of reducing C loss. However, there is a wealth of evidence to show that this intuitive solution may not have the desired effect.

Firstly, NT treatments may not actually lead to increases in SOC storage, but in fact to a redistribution of SOC in the soil profile (Ball *et al*, 1996; Morell *et al*, 2011; Sun *et al*, 2011; Van den Putte *et al*, 2012) as crop residue (fresh OM) is left on the soil surface rather than being incorporated deeper in the soil (Ball *et al*, 1996). Ball *et al* (1989) continued an experiment monitoring the effects of different long term tillage treatments. They analysed the SOC content under each of these treatments to a depth of 18 cm after 22 years of treatment and found that although there was no difference in the total SOC under NT (direct drilling) and CT plots, the SOC was more concentrated near the surface under NT (Ball *et al*, 1996). Powlson *et al* (2012) found statistically insignificant increases of C in a review of zero tillage experiments in the UK ($310 \pm 180 \text{ kg C ha}^{-1} \text{ yr}^{-1}$). Andruschkewitsch *et al* (2013) examined long-term (18 to 25 years of treatment) NT and CT plots at four sites. They found higher concentrations of unprotected and aggregate occluded OM, and macro-aggregate concentrations in the top 5 cm of NT plots compared to CT plots but from 5–25 cm the aggregate occluded OM was higher in the CT plots. The total SOC concentrations under the different treatments at all depths were not significantly different, i.e. the SOC was merely distributed differently across depths and OM fractions in the two treatments.

Although macro-aggregates form at the same rate under CT and NT (Six *et al*, 1999a), those under CT are less stable than those under NT (Beare *et al*, 1994; Ball *et al*, 1996) and can be broken up during tillage, particularly larger aggregates, (Jordán *et al*, 2010) so preventing the formation of micro-aggregates, and reducing the physical protection of SOC (Six *et al*, 2000). Carter (1992) found that aggregates under NT (0–5 cm) had slightly higher SOC contents than CT, particularly those that were 1–2 mm in size. Six *et al* (1999a) found that although there was no significant difference in the total SOC (0–5 cm) found in

macro-aggregates of NT and CT soils, there was three times more in the highly stabilised micro-aggregates of the NT. As macro-aggregates under CT have a turn-over rate up to twice as high as under NT (Six *et al*, 2000) and crop residues are incorporated deeper into the soil, a high proportion of SOC is made available to the decomposer community relatively quickly (Jacobs *et al*, 2010). In this way CT leads to a loss of C-rich macro-aggregates and an increase in C-depleted micro-aggregates and an overall loss of SOC from the soil. (Six *et al*, 2000). Plaza *et al* (2013) found that microbially derived organo-mineral complexes within micro-aggregates were the most important stabilisation mechanism in NT soils providing both physical and chemical protection of SOC (i.e. adsorption to mineral surfaces and entrapment of POM).

In a paired plot experiment comparing the effects of 10 years of either CT or NT, Freixo *et al* (2002) found that a greater proportion of whole soil C was stored in unprotected POM in the 10–20 cm layer in CT soils than under NT soils (3.8% and 1.4%, respectively). Denef *et al* (2004) found greater proportions of whole soil C associated with micro-aggregates under NT than CT. Differences in micro-aggregate associated C explained 90% of the difference in total C between CT and NT (Denef *et al*, 2004), whilst Huang *et al* (2010) found that ~45% of the difference was accounted for by micro-aggregate C in a similar study. Du *et al* (2015) observed increased SOC associated with minerals in NT and reduced tillage systems than in CT.

The breaking up of aggregates by tillage stimulates microbial respiration in the soil, and so greater CO₂ emissions, by making SOC physically available (Roberts and Chan, 1990). Therefore, reducing tillage will decrease this loss of SOC through microbial decay. Ball *et al* (1999) observed decreased CO₂ and increased N₂O emissions under NT after rainfall, probably due to increased water content producing anaerobic conditions. However, this effect is highly variable between sites (Rochette, 2008).

When discussing the impacts of agricultural land management practices on SOC it is also important to consider the implications for other GHG gases which have a greater impact on the atmosphere than CO₂: N₂O and CH₄. N₂O is produced naturally in soils through the microbial processes of nitrification (oxidation of ammonia) and denitrification (reduction of nitrate) (Cloy and Smith, 2015). Ball *et al* (1999) found that N₂O emissions increased markedly in NT treatments after rainfall, likely due to a reduction in hydraulic conductivity causing higher water contents in the topsoil (Van den Putte *et al*, 2012). This low aeration in poorly drained soils favours denitrification. Rochette (2008) conducted a meta-analysis of N₂O emissions data from 25 sites with NT and CT treatments (collectively giving 45 years of measurements). He found that emissions were generally higher from NT plots, and particularly when the plots were poorly drained (less aerated), with N₂O emissions being on

average 2 kg N ha⁻¹ (1.5 times) greater than CT plots at the same site. Conversely NT plots on well drained sites emitted only 87% of that emitted from their CT equivalents.

Methane is formed in soils by a process called methanogenesis which is the microbial breakdown of organic compounds in strictly anaerobic conditions. Several bacteria that degrade organic material via a complex food web are needed to perform methanogenesis. The final step is performed by methanogens, methane producing bacteria. The phenomenon of CH₄ oxidation by soil bacteria is general in aerated soils. In well-drained and therefore well-aerated soils, in the absence of termites, any CH₄ present is largely that which has diffused in from the atmosphere. Methane is oxidized to CO₂ by methanotrophic bacteria which derive their energy from the oxidation of CH₄, consequently this means that aerobic soils are normally sinks of CH₄ (Cloy and Smith, 2015). Disturbance by tillage decreases the soil diffusivity through loss of structure and porosity. Ball *et al* (1999) calculated a 75% reduction in CH₄ oxidation, compared to NT, after ploughing to 20 cm. As a result of detrimental effects of soil disturbance and additions of nitrogenous fertilizers on methanotrophic organisms, conversion of natural soils to agricultural use reduces CH₄ oxidation rates. Recovery of oxidation rate from land-use change or fertilization is slow; it may take 100 years or more to return to pre-disturbance rates (Cloy *et al*, 2015).

1.2.2 Inorganic N Fertilisation

Alvarez (2005) conducted a meta-analysis of 111 studies of N fertiliser treatments versus control plots and found that the addition of inorganic N fertiliser to soils has been shown in different studies to increase (88 studies), decrease (16 studies) (all studies in this category had applications rates < 1 t N ha⁻¹) and have no impact (7 studies) on the accumulation rate of SOC. Alvarez (2005) also found that as the rate of N application increases, so too did the SOC sequestration rate, by around 2 t C ha⁻¹ for each additional 1 t N ha⁻¹. Increases in SOC due to the addition of inorganic N fertiliser are generally modest and decrease through time as the soil reaches a new equilibrium (Powlson *et al*, 2011). The addition of inorganic N fertiliser was found to have no effect on aggregation (Yu *et al*, 2012). Bhattacharyya *et al* (2010) observed only a small increase in percentage of macro-aggregates after 30 years of FYM and inorganic fertiliser additions.

These variations in the effect of N fertilisation on SOC could be due to the light (labile) and heavy (organo-mineral) soil fractions reacting in opposing ways. Neff *et al* (2002) used biomarkers to trace the fate of OC inputs to soil under N fertilisation on a Canadian alpine meadow and found that the light fraction is decomposed more rapidly under long term N fertilisation, whilst a greater proportion of relatively un-decomposed SOC is stabilised in the heavy fraction. These alterations in C turnover times ultimately had no effect on the total SOC pool. Conversely, Khan *et al* (2007) reported net losses of SOC in the soil profile, with

losses in the sub-surface soil being greater than the upper layer for an N fertilised silt loam in the USA that was monitored for 51 years, a finding supported by a large body of literature. Khan *et al* (2007) assert that studies which show increases in SOC due to N fertilisation are flawed through the comparison of fertilised plots to unfertilised controls rather than tracking treated plots through time and comparing to baseline data. Observed increases in SOC are due to increased crop yields leading to higher returns of residue to the soil, so if these residues are removed then the addition of N has no effect on SOC (Alvarez, 2005).

Any apparent SOC benefits from N fertilisation are also overshadowed by the GHG emissions produced during the manufacturing and application of the fertiliser, which are almost four times greater than the additional SOC sequestered (Powlson *et al*, 2011). This means that in real terms the addition of inorganic fertiliser could still lead to a net negative effect for the climate despite any increase in soil C sequestration (Alvarez, 2005; Powlson *et al*, 2011).

Carbon dioxide emissions are indirectly affected by N fertilisation through the stimulation of root growth, and consequently greater root respiration (Inselsbacher *et al*, 2011). N fertiliser has been shown in laboratory studies to reduce microbial respiration, with the reduction being greater with higher concentrations of N added (Ramirez *et al*, 2012). This is possibly because N limitation is removed and the microbial community shifts towards one which utilises more labile SOM, rather than one which uses labile C as an energy source to ‘mine’ recalcitrant SOM to obtain N so reducing overall microbial respiration activity (Ramirez *et al*, 2012).

Inorganic N fertiliser can increase CO₂ and N₂O emissions from the soil and decrease or reverse its CH₄ sink capacity (Inselsbacher *et al*, 2011). In an incubation study Inselsbacher *et al* (2011) found that, four hours after N application, N₂O emissions were elevated compared to controls for three days, with a second application eight days later showing an even stronger increase in emissions despite 25% less fertiliser being applied. Interestingly, a greater proportion of the N₂O-N released after the second application originated from the fertiliser rather than the soil N stock, although the overall loss of fertiliser N as N₂O was minimal (0.3%). Fertilisation temporarily creates an excess in the soil of the polyvalent ions required for microbial nitrification and denitrification (NH₄⁺ and NO₃⁻ respectively) which reduces microbial competition for these resources (Norton and Firestone, 1996). The concentration of N₂O in soil air has also been observed to increase (Hansen *et al*, 1993).

1.2.3 Interaction between tillage and inorganic fertiliser

Poirer *et al* (2009) found interacting effects between tillage and inorganic N fertilisation in the top 20 cm of a clay loam in Canada. They applied different rates of N fertiliser to both

CT and NT plots and found that high N application rates decreased SOC storage in CT plots and increased it in NT plots. Similar results were observed by Morell *et al* (2011). Poirer *et al* (2009) attribute this to excess N activating N limited microbes, and so increasing mineralisation in the CT plots and residue accumulation at the surface of NT plots. When comparing SOC stocks down to 60 cm there was no difference in volume of SOC between any of the treatments, likely due to the redistribution of SOC.

1.2.4 Organic fertiliser

Organic fertiliser differs from inorganic fertiliser in that it is a return of non-manufactured nutrients and biomass derived C to the soil, although it is unlikely to be returned to the soil from which it originated, and so there is a much lower GHG cost associated with its production.

Incubations of soils which had had long term NPK fertiliser and livestock manure added had higher respiration than that with just NPK, but also had the largest labile C pool and the highest aggregate stability (Cheng-Liang *et al*, 2012). Yu *et al* (2012) observed a 124% increase in SOC with compost fertilisation compared to unfertilised plots on a sandy loam soil in China, whilst a plot fertilised with a half organic compost and half inorganic N mixture had a 72% increase (still a 45% higher increase than inorganic fertiliser alone). Similar results have been observed by Bhattacharyya *et al* (2010) after 30 years of farmyard manure (FYM) and inorganic fertiliser mix applications to a sandy loam in India (40% greater in the upper 45 cm than inorganic fertiliser alone). Conversely, Rasool *et al* (2008) found that inorganic fertiliser caused a greater increase in SOC than FYM after 32 years of treatment on a different sandy loam in India. Rasool *et al* (2008) speculate that this may be due to a greater accumulation of root biomass in the inorganically fertilised plots. Liang *et al* (2012) found that after 15 years of fertiliser treatments on a silty loam in China, soil fertilised with FYM had significantly higher SOC stocks from 0–30 cm depth and significantly higher particulate OM from 0–20 cm depth when compared to unfertilised and inorganic fertilised plots. Finally, in a review of SOC increases in UK soils, Powlson *et al* (2012) reported increases of 20–100 kg C ha⁻¹ yr⁻¹ t⁻¹ dry matter added, dependent on the type of organic amendment, with an average increase of 60 kg C ha⁻¹ yr⁻¹ t⁻¹ dry matter. However, the rate of C sequestration is known to decrease with the time over which organic amendments are applied as a new equilibrium is reached (Powlson *et al*, 2012).

In a different meta-study it was found that the increase in SOC stock relative to a control with no organic amendment had a linear relationship to the amount of amendment C added across the whole experimental time frame (Millard and Angers, 2014).

The addition of organic fertiliser has been observed to increase the proportion of macro-aggregates and to decrease the proportion of micro-aggregates and the free silt and

clay fraction (Bhattacharyya *et al*, 2010, Yu *et al*, 2012). Yu *et al* (2012) found a 250% increase with organic compost fertilisation, a 101% increase with half compost, half inorganic N fertiliser mix and no significant difference with inorganic fertiliser alone when compared to plots with no fertilisation. Furthermore, these macro-aggregates were C enriched compared to the other fractions, with C accumulating in the silt and clay sub-fraction. Increases in micro-aggregate C occurred in the more labile intra-aggregate LF. However, Bhattacharyya *et al* (2010) found the C accumulation rate to decline with aggregate size.

1.2.5 Biochar

Pyrolysis of biomass (slow heating of OM to high temperatures under low oxygen conditions) creates a highly stable, C rich by-product termed ‘biochar’ (Sohi *et al*, 2009). There has been much research in recent years into the possibility of using biochar as an agricultural soil amendment and as a long term C sink (Glaser *et al*, 2002, Laird, 2008; Novak *et al*, 2009)

The suitability of biochar to perform these two functions lies in its ability to improve soil quality by increasing water holding capacity, reducing bulk density, improving fertility, increasing pH and increasing nutrient retention (Glaser *et al*, 2002). Lehmann *et al* (2011) points out that it has not been made clear in the literature whether these benefits are brought about by distinct changes in the soil surrounding biochar or if they “are merely an average of soil and biochar properties”. Biochar has a high OC content and is resistant to microbial decay; carbonized OM in the *Terra preta* of the Amazonian basin has been dated at 1000–1500 years old (Glaser *et al*, 2000).

Newly formed (young) biochar has a labile fraction which decomposes rapidly when initially added to soil. Smith *et al* (2010) observed increased CO₂ emissions after biochar addition. This CO₂ was of biochar origin and the increase was greater with increased biochar addition, but only lasted for six days after the addition of biochar giving strong evidence for a labile fraction.

There have been contradictory reports of the effects of biochar addition on N₂O emissions. Yanai *et al* (2007) found that the response depended on the activity of the denitrifying microbial community as a consequence of the water filled pore space (WFPS) of the soil. When re-wetted to 73% WFPS, N₂O emissions in charcoal amended soils were suppressed by 89% compared to soil with no charcoal added and at 83% WFPS, N₂O emissions were increased by 51% in charcoal amended soils compared to soils without charcoal addition.

Research has concentrated on the effects of biochar on soil ecology, crop yield, crop disease and soil C dynamics, but relatively little is known about the effects of biochar on SOC

stability and macro-aggregation (Sohi *et al*, 2009). Brodowski *et al* (2005) fractionated soil containing black C, they found that it had formed associations with minerals (both small particles of black C bound to minerals and large particles of black C with minerals bound to it as well as free particles with associated minerals) that they concluded could not be incidental and were due to chemical and physical organo-mineral interactions. Chen *et al* (2011) used thermodynamic analysis to show that biochar adsorption of minerals was not a passive reaction. Black C is by its nature very light; Brodowski *et al* (2005) hypothesise that the presence of black C in the heavy fraction may be due to its initial high rate of decomposition facilitating interaction with soil minerals and this in turn protects the black C from further decomposition. The O:C of black C increases as it rapidly decomposes, producing increased numbers of highly aromatic O-functional groups (such as carboxylic and hydroxylic groups). These small, light black C particles are then able to combine with minerals in the medium and heavy fractions (Brodowski *et al*, 2005).

This association with minerals helps to improve soil aggregation. Mbagwu and Piccolo (1997) found that small amounts of coal-derived humic acids added to soil achieved a 20–130% increase in aggregation where 33–133 times more organic residue was required to gain the same effect. George *et al* (2012) observed positive effects of hydrochar (biomass suspended in water and then heated to high temperatures) on aggregation. Conversely Busscher *et al* (2010) found no effect of biochar on aggregation when added to coastal agricultural soils in the USA. However, they do note that this may be a consequence of the feedstock and pyrolysis methods used and they expect the carbon will still have a long residence time. The hydrophobic, polyaromatic structure of biochar prevents water from entering macro-aggregate pores and so increases their stability and turn-over time (Glaser *et al*, 2002).

1.3 Land-Use Change

Land-use has a big effect on the SOC storage capacity of soils, and as such they are not always storing C to their maximum capacity. This could then be thought of as ‘unused capacity’ for SOC storage which could be ‘topped-up’ by changing land-use. Poeplau and Don (2013) found that subsoil (below 30 cm) OC stocks were higher under cultivation than a permanent grassland due to fresh C being directly input to this layer through incorporation of residues, a result they note also observed by Rasse *et al* (2006), Huggins *et al* (2007) and Don *et al* (2009). With the cessation of ploughing and conversion to grassland at this site, the subsoil OC stock declined, and was still declining more than 40 years later, suggesting that the grassland sub-soil had not yet reached a new equilibrium. However, they did not observe this effect in the other five sites studied.

Similarly, the conversion of permanent grassland to cropland results in a loss of SOC. Poeplau and Don (2013) calculated SOC losses from six such sites across Europe and found that there was a loss of $23 \pm 5 \text{ Mg C ha}^{-1}$ in the top 30 cm, which may have all been lost in the 17 years following the first tillage (Poeplau *et al*, 2011). Conversely, six former croplands which had been converted back to grassland only accumulated $17 \pm 5 \text{ Mg C ha}^{-1}$ in the upper 30cm, only two thirds of the average lost. This provides evidence that C stocks in top soils cannot simply be ‘topped back up’ through land-use change and it may take in excess of 100 years for such soils to reach their full sequestration potential (Poeplau *et al*, 2011).

The soil mineralogy also plays a part in the effect that tillage has on SOC. A meta-analysis of 45 studies of grassland to cropland conversion showed that, after time since conversion, clay content was the most important explanatory variable for the rate of SOC loss (Poeplau *et al*, 2011). This is likely due to there being a greater proportion of SOC chemically protected through association with clay minerals.

1.4 Greenhouse gas emissions from agricultural soil

Greenhouse gases do not impact on the climate equally and are commonly expressed on a CO₂ equivalent basis for comparison. When calculated in this way the global warming potentials over a 100 year period of N₂O and CH₄ are 298 and 25 times more potent than CO₂, respectively. The previous sections have outlined how specific agricultural land management practices affect fluxes of GHGs.

Greenhouse gas fluxes from agricultural soils are influenced by environmental factors such as temperature and precipitation, soil physical factors such as texture (which affects pore space distribution and gas diffusivity) (Smith *et al*, 2003), soil chemical properties such as pH, nutrient availability and mineralogy (Batjes and Bridges, 1992) and biological factors such as the microbial community structure (Morales *et al*, 2010).

1.5 Aims and objectives

The general aims of this research are to investigate the impacts of different agricultural land use and land management practices on the stability of SOC and to assess the relative importance of different stabilisation mechanisms under different management conditions (particularly tillage and managed grassland). Chapter 2 was a preliminary study, the objectives of which were to determine the impacts of inorganic fertiliser and soil moisture on GHG emissions from a range of arable and grassland soils which were chosen to be representative of the most common agricultural areas in the UK.

The objectives of Chapter 4 were to assess the comparability of soil fractionation methods which isolate SOM fractions with different chemical and physical properties and/or C turnover rates, and to test if the simplest method could be used as an indicator of soil C stability. The results of this chapter were used to inform the choice of fractionation method for the following two chapters.

The objectives of Chapter 5 were to assess the changes in SOC stocks and relative importance of different C stabilisation mechanisms, using soil fractionation, after a land-use change from arable to grassland, utilising an abandoned long-term tillage experiment which had plots under various tillage management treatments.

Chapter 6 further investigates the impacts of tillage on SOC stability by directly assessing the vulnerability of SOM to decomposition after the removal of physical protection through fractionation. A further objective was to assess the temperature sensitivity of isolated SOM fractions and intact soil.

2 Factors controlling greenhouse gas emissions from a representative range of UK agricultural soils

2.1 Introduction

Agricultural soils are important sources of atmospheric greenhouse gases (GHGs). Various soil properties and environmental factors have complex interactions which influence the dynamics of these GHG fluxes. Soil texture is a particularly important factor as it dictates soil water dynamics, pore space and gas diffusivity which in turn affect microbial activity and the rate at which GHGs are emitted from, or taken up by, the soil. Soil texture, particularly clay content, also influences the stability of soil C; higher concentrations of clay minerals provide greater opportunity for chemical protection by adsorption of organic matter (OM) to clay particle surfaces (Oades, 1988), and so prevent its decomposition by soil microbes and subsequent emission as CO₂ from the soil.

Soil GHG fluxes are particularly sensitive to water filled pore space (WFPS). Soil CO₂ emissions decrease substantially after heavy rainfall because high WFPS and poor gas diffusivity and air-filled porosity restrict respiration and increase anaerobic conditions (Ball *et al*, 2013a; Ball *et al*, 2013b). For soils with different clay contents, Franzluebbers (1999) found that cumulative C mineralization during a 24 day incubation at 25 °C increased with increasing WFPS to a maximum of 0.53–0.66 m³ m⁻³. Clay content had no effect on the level of WFPS required to achieve maximum C mineralization under compressed and uncompressed conditions (Franzluebbers, 1999). As for CO₂, emission of N₂O by nitrification increases linearly with increasing soil water content to a maximum of 60% WFPS and then decreases (Shepherd, 2009). While the WFPS needs to be 60–65% for substantial emissions of N₂O to occur (i.e. critical WFPS), the highest emissions occur by denitrification when the WFPS is between 70 and 90% with lowest at WFPS < 50% (Shepherd, 2009). The critical WFPS is a major driver of GHG emissions and in finer textured soils the critical WFPS and the subsequent degree of saturation required to generate GHGs decreases so that these soils tend to emit more GHGs than coarser textured soils. The application of fertiliser N has the biggest impact on soil N₂O production but blockage of the air-filled pores by water near the surface of compacted soils can dramatically increase N₂O emission and decrease CO₂ emission (Ball, 2013a; Ball *et al*, 2013b). Production of CH₄ via methanogenesis requires anaerobic soil conditions and specific redox conditions, and methanotrophy (CH₄ oxidation to CO₂) requires aerated soils to facilitate diffusion of CH₄ into the soil from the atmosphere (Cloy and Smith, 2015).

Nitrous oxide fluxes from agricultural soils have been shown to be sensitive to soil properties such as pH, WFPS (Weslien *et al*, 2009; Bouwman *et al*, 2002) and available C

(Bouwman *et al*, 2002) as well as environmental factors such as temperature and precipitation (Dobbie *et al*, 1999; Hinton *et al*, 2015) and management practices including fertiliser application and crop rotation (Bouwman *et al*, 2002). The effects of soil texture on N₂O emissions have been found to be both significant (Maag and Vinther, 1996) and insignificant (Skiba and Ball, 2002).

Application of ammonium nitrate (AN) fertiliser to soil temporarily creates an excess of the polyvalent ions required for microbial nitrification and denitrification (NH₄⁺ and NO₃⁻, respectively) which reduces microbial competition for these resources (Norton and Firestone, 1996). Studies have shown an increase in soil N₂O fluxes after AN fertiliser application to agricultural soils (Bouwman *et al*, 2002; Clayton *et al*, 1997; Inselsbacher *et al*, 2011; Hinton *et al*, 2015; Bell *et al*, 2015b). Use of AN fertiliser may also increase CO₂ emissions from the soil and decrease or reverse its CH₄ sink capacity (Inselsbacher *et al*, 2011).

It would be expected that increasing soil moisture content above critical threshold levels would cause an increase in N₂O and CH₄ emissions due to an increase in anaerobic micro-sites in soil pores, and an accompanying decrease in CO₂ as soil respiration is a strictly aerobic process. The level at which these micro-sites are created will depend on the porosity of the soil. Fine textured soils will have smaller pores which will fill up at lower moisture contents than coarse textured soils.

The purpose of this study was to assess the differences in GHG fluxes from arable and grassland agricultural soils which represent the range of soil textures and climatic conditions of the main agricultural areas in the UK, under different moisture contents and with or without inorganic fertiliser application. It was hypothesised that 1. soils at higher moisture contents would have significantly higher N₂O and CH₄ fluxes and lower CO₂ fluxes, than soils at lower moisture levels because oxygen supply would be more limited, 2. Coarse textured soils would have significantly lower N₂O and CH₄ emissions and higher CO₂ emissions than fine textured soils due to a lower number of saturated soil pores and 3. N-fertilised soils would release more N₂O and CO₂ than non-fertilised soils as N limitation on the microbial community will be alleviated.

2.2 Materials and Methods

2.2.1 Soils

Soils were collected to a depth of 10 cm from nine sites (four arable and five grassland), sieved to < 4 mm, air dried and stored in sealed plastic bags. These sites were carefully chosen to represent the different soil types and climates of the main agricultural areas across the UK. The soils collected from field sites which were part of the Defra funded Agricultural Greenhouse Gas (GHG) Research Platform. One of the platform aims is to improve the resolution and accuracy of reported N₂O (project AC0116) emissions from agriculture and the factors affecting these. Field plot scale experiments measuring N₂O emissions from grassland and arable sites after inorganic N, different types of manures and animal excreta application have been undertaken as part of the AC0116 project. This Chapter will focus on inorganic N application under controlled conditions using soil from selected AC0116 field sites. However, results from Boghall (Scottish AC0116 arable site) after organic fertiliser application and Crichton (Scottish AC0116 grassland site) after animal excreta application are presented in Appendix 1. Table 2.1 gives a summary of climatic and soil properties for the AC0116 sites used in this experiment and Figure 2.1 shows a map of the field site locations. More details about the GHG platform can be found at <http://www.ghgplatform.org.uk>.

Table 2.1: Land use, average annual temperature (°C), soil texture and annual average precipitation for the nine soils

Land Use	Site	Average annual temp. (°C)	Soil texture	Annual precipitation (mm)
Grassland	Crichton	10.2	Sandy loam	> 950
	Drayton	10.3	Clay	0 - 750
	Hillsborough	9.8	Clay loam	751 - 950
	North Wyke	9.6	Silty clay	> 950
	Pwllpeiran	9.3	Clay loam	> 950
Arable	Gilchriston	8.7	Sandy Clay loam	< 750
	Woburn	10.9	Loamy sand	< 750
	Rosemaund	10.4	Clay loam	751 - 950
	Boxworth	9.7	Clay	550

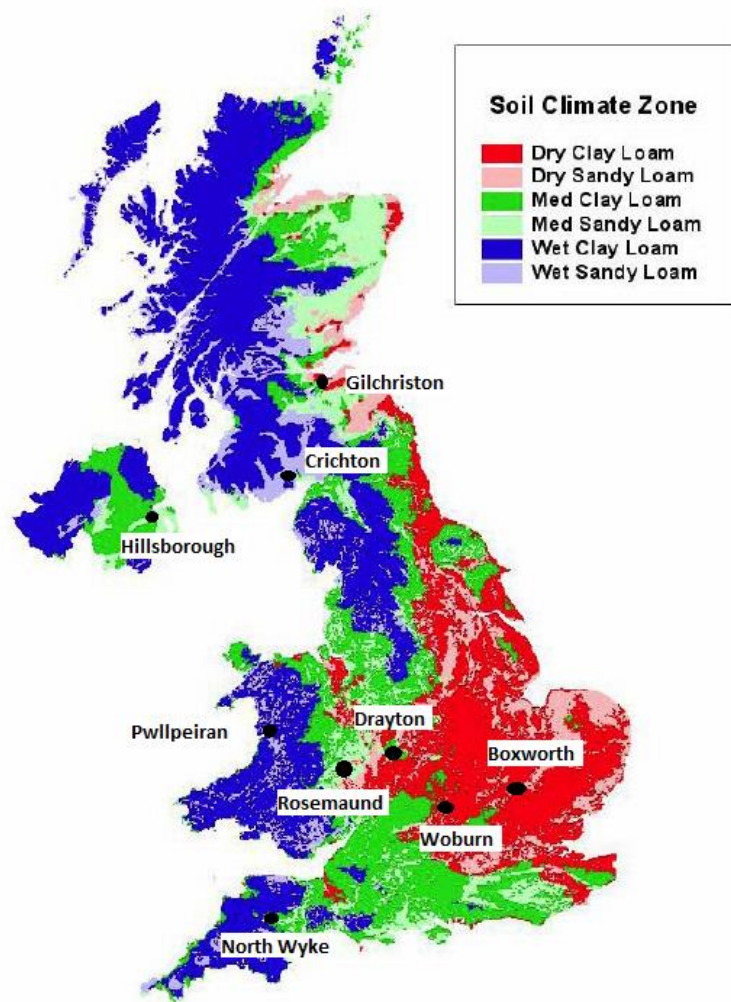


Figure 2.1: Map showing the locations of the field sites.

2.2.2 Treatments

A fully-factorial experiment was designed with two moisture levels (50% and 80% water holding capacity (WHC)) and two ammonium nitrate (AN) application levels (0 or 70 kg ha⁻¹). Soils had been sieved to < 4 mm, air-dried and stored for at least two years. WHC was used instead of water filled pore space (WFPS) in this study as the soils no longer had any structure; when repacked, volumes of water to be added to adjust to 50 or 80% WFPS (calculated using field bulk density values) proved to be consistently too high for all soils, resulting in waterlogging. The AN was applied on an area basis to replicate field applications.

Following the method of Howard and Howard (1993), WHC was calculated by placing 10 g of dry soil (n = 3 for each soil) in a plastic funnel plugged with glass wool and saturating the soil with deionised water. The funnel was then covered with plastic film to minimise evaporation and left to drain for 4 hours (or until no more water was observed dripping from the funnel). The soil was then placed in pre-weighed tin trays, wet masses recorded, then

oven-dried overnight at 107°C and reweighed. The volume of water at 100% WHC was then calculated using the following equation:

$$100\% \text{ WHC} = \frac{\text{mass saturated soil} - \text{mass oven dry soil}}{\text{mass oven dry soil}}$$

Treatments were applied to 80 g of dry soil ($n = 3$) in 500 ml Kilner jars fitted with two three-way tap sampling ports. Deionised water was applied evenly to the soil by gently spraying it on and lightly shaking the jar to bring dryer soil to the surface, in sufficient volume to adjust the dry soil to the appropriate WHC. Fertiliser was applied by dissolving AN in the deionised water used to adjust the moisture content of the air dried soil, ensuring even distribution of AN throughout the soil. Soils were carefully packed to a bulk density of $\sim 1 \text{ g cm}^{-3}$, which was the average value found from field measurements of the Defra InveN₂Ory soils (ranging from 0.6–1.6 g cm^{-3}). From this point treatments will be referred to as 50, 80, 50+N or 80+N to denote 50% WHC, 80% WHC, fertilised 50% WHC and fertilised 80% WHC, respectively.

Soils were incubated at 10 °C (this temperature was chosen as it is close to the average annual temperature for all of these sites, see Table 2.1) for either 22 or 23 days in batches of one or two sites.

2.2.3 Headspace gas sampling

Headspace gas samples were taken from the Kilner jars twice per week at the beginning (t_0) and end (t_1) of a one hour closure period. Before each sampling period, jars were opened and allowed to sit for 3 minutes to allow gas concentrations in the jar to equilibrate with the laboratory air before sealing the lids with both sampling ports in the open position. The jars were then flushed three times through one of the sampling ports using a 60 ml syringe before drawing t_0 gas sample and injecting it into a 25 ml pre-evacuated vial, after which both ports were immediately closed. Leaving both ports open during the t_0 sampling prevents a negative pressure being created within the jar which could cause gas to be drawn out of the soil pores, leading to over estimation of fluxes.

After the t_0 headspace gas sampling, jars were returned to the incubator and the t_1 samples were drawn one hour later from one of the ports (the other remained closed). Between sampling periods jar lids were closed and both ports remained open to allow free gas exchange within the incubator whilst limiting moisture loss. The mass of all Kilner jars were recorded at the beginning and end of incubations to calculate moisture loss from soils, mass loss was never more than $\sim 1\%$, and so moisture loss from soils was not deemed to be significant.

Headspace CO₂ concentrations were used to calculate CO₂-C fluxes per day (mg CO₂-C day⁻¹) using linear regression (linear increase in CO₂ concentration in a known volume over a fixed period) and the ideal gas law (Saggar *et al*, 2008) as follows:

$$F = \rho \frac{V}{A} \frac{\Delta C}{\Delta t} \frac{273.15}{T + 273.15}$$

This calculation assumes a linear increase in gas concentration in a known volume over a known period of time, where F is the flux, ρ is the density of the gas, V is the volume of the jar, A is the basal area of the jar, ΔC is the difference between the gas concentrations at t₁ and t₀, Δt is the jar closure time in hours, T is the incubation temperature in °C..

Cumulative fluxes were calculated using the trapezoidal rule (area under the curve) to interpolate fluxes between sampling days (Hinton *et al*, 2015) as follows:

$$\text{Cumulative flux} = (\text{day } x \text{ cumulative flux} + \text{day } y \text{ flux}) + (\text{mean}(\text{day } x \text{ flux} + \text{day } y \text{ flux})) \\ * (\text{day } y - \text{day } x - 1)$$

Emission factors (EFs) define the percentage of applied N fertiliser which is emitted as N₂O. The Intergovernmental Panel on Climate Change (IPCC) Tier 1 methodology for calculating N₂O emissions from agricultural soils assumes an EF of 1% (IPCC, 2006a). EFs were calculated for N₂O emissions from AN fertilised soils using the following equation:

$$EF = \left(\frac{\text{Cumulative FN}_2\text{O flux (kg N}_2\text{O} - \text{N}) - \text{Cumulative CN}_2\text{O flux (kg N}_2\text{O} - \text{N})}{N \text{ applied (kg N)}} \right) * 100$$

where FN₂O is the N₂O flux from AN fertilised treatment at either 50 or 80% WHC and CN₂O is the N₂O flux from the unfertilised control at either 50 or 80% WHC.

2.2.4 Analysis

Pre- and post-incubation soil mineral N concentrations were determined. Soil subsamples were extracted with 2 M KCl (soil to KCl ratio 1:2) within 24 hours of the final headspace gas sampling. Extracts were analysed for NH₄⁺-N and NO₃⁻-N using a Skalar San⁺⁺ continuous flow colorimetric autoanalyser (Skalar, York, UK). The analyser was calibrated using 0, 2, 4, 6, 8 and 10 mg L⁻¹ NH₄⁺-N and 0, 1, 2, 3, 4 and 5 mg L⁻¹ NO₃⁻-N + NO₂⁻-N. Colorimetric determination was carried out at wavelengths of 650 nm and 540 nm for NH₄⁺-N and NO₃⁻-N, respectively, following the methods of Singh *et al* (2011).

Pre- and post-incubation soil subsamples were also extracted with deionised water (1:2 ratio for 60 minutes, allowed to settle for 30 minutes) and soil solution pH was measured using a calibrated pH electrode (Thermo-Orion, Beverly, MA, USA).

Air dried and ball milled soil samples were combusted and analysed for organic C (OC) and total nitrogen (TN) using a Flash 2000 elemental analyser. The chemical characteristics of these soil samples were also determined using a Bruker Vertex 70 Fourier Transform Infrared (FT-IR) spectrometer using a Diamond Attenuated Total Reflectance accessory (Bruker Optics, Ettlingen, Germany).

2.2.5 Statistical Analysis

To test for within soil differences between WHC and AN treatments, statistical analyses of cumulative GHG fluxes, pre- and post-incubation pH and mineral N contents was carried out using two-way ANOVA, with a confidence level of $p < 0.05$. Significant differences between EFs for different soils were determined by one-way ANOVA ($p < 0.05$), EFs at 50 and 80% WHC were analysed separately. For between soil differences, the grassland and arable soils were analysed separately by multiple linear regression to evaluate the influence of % clay (as a proxy for soil texture), % OC, % TN, C:N, pH and $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ content. Reduced models were determined by backwards selection of the most significant variables. All statistics were carried out using Genstat (15th edition).

2.3 Results

2.3.1 Soil properties

Average TN, OC and clay contents, C:N and pH ranged from 0.27–0.77%, 2.22–12.35%, 15.0–56.5%, 8.17–15.99 and 4.47–6.67, respectively for the grassland soils, and 0.09–0.77%, 0.94–1.90%, 11–45%, 8.21–13.57 and 5.13–7.91, respectively, for the arable soils (Table 2.2). These differences in soil properties are due to natural variation in geology, topography and climate.

6.3.2 N_2O fluxes

Nitrous oxide flux data was log transformed after adding 1.025 to all data points (to eliminate negative values), forcing the data to more fully satisfy the assumption of normal distribution of the residuals.

Generally, WHC and AN treatments had no significant impact on arable soil cumulative N_2O fluxes (Figure 2.2a). The exceptions were the 80+N treatments for Boxworth and Gilchriston which were significantly higher ($p < 0.001$) than the other treatments in their respective soils. Gilchriston fluxes were particularly high, up to 107 times greater than other soils for the 80+N treatment ($46 \pm 9 \text{ mg N}_2\text{O-N m}^{-2}$), and so are plotted on a separate scale. Rosemaund

was a net sink of N₂O for all treatments (-0.2 ± 0.3 to -0.3 ± 0.4 mg N₂O-N m⁻²). At 50% WHC, addition of fertiliser suppressed cumulative N₂O emissions (increased the sink for Rosemaund soil) by 0.03–0.97 mg N₂O-N m⁻², except for Gilchriston where there was an increase of 0.4 mg N₂O-N m⁻².

The 80+N treatment cumulative N₂O fluxes from the grassland soils were significantly higher compared to other treatments of the same soil in all cases except Pwllpeiran ($p < 0.005$ for Drayton and < 0.05 for North Wyke and Crichton, two-way ANOVA) (Figure 2.2b). N₂O emissions from the Crichton soil increased significantly ($p < 0.05$) from 3.3 ± 1 to 49 ± 18 mg N₂O-N m⁻² between the 50 and 80 treatments and from 1.9 ± 0.5 to 69 ± 26 mg N₂O-N m⁻² between the 80 and 80+N treatments. Fluxes were particularly high for the Crichton soil with 80+N treatment fluxes up to 244 times greater than fluxes from the Pwllpeiran soil.

Table 2.2: Pre-incubation average 100% water holding capacity (WHC), total nitrogen (TN), organic carbon (OC), inorganic carbon (IC), C:N, clay content (% by weight) and pH for the grassland and arable agricultural soils.

Land Use	Site	100% WHC (g water g ⁻¹ soil)	TN (g N kg ⁻¹ soil)	OC (g C kg ⁻¹ soil)	IC (g C kg ⁻¹ soil)	C:N	Clay (% by weight)	pH
Grassland	Crichton	0.55	3.058	32.6	0.6	10.68	12.5	5.3
	Drayton	0.51	4.616	50.8	6.1	11.01	50.1	6.7
	Hillsborough	0.34	7.723	12.4	0.5	15.99	28.1	5.9
	North Wyke	0.64	2.718	22.2	0.3	8.17	32.5	4.5
	Pwllpeiran	0.7	4.408	41.8	0.3	9.49	23.8	5.1
Arable	Gilchriston	0.45	0.932	12.7	0.1	13.57	12.7	5.9
	Woburn	0.45	0.861	9.41	0.3	10.93	10.0	7.0
	Rosemaund	0.30	1.282	10.5	0.2	8.21	20.9	5.1
	Boxworth	0.51	1.854	19.0	1.5	10.27	44.8	7.9

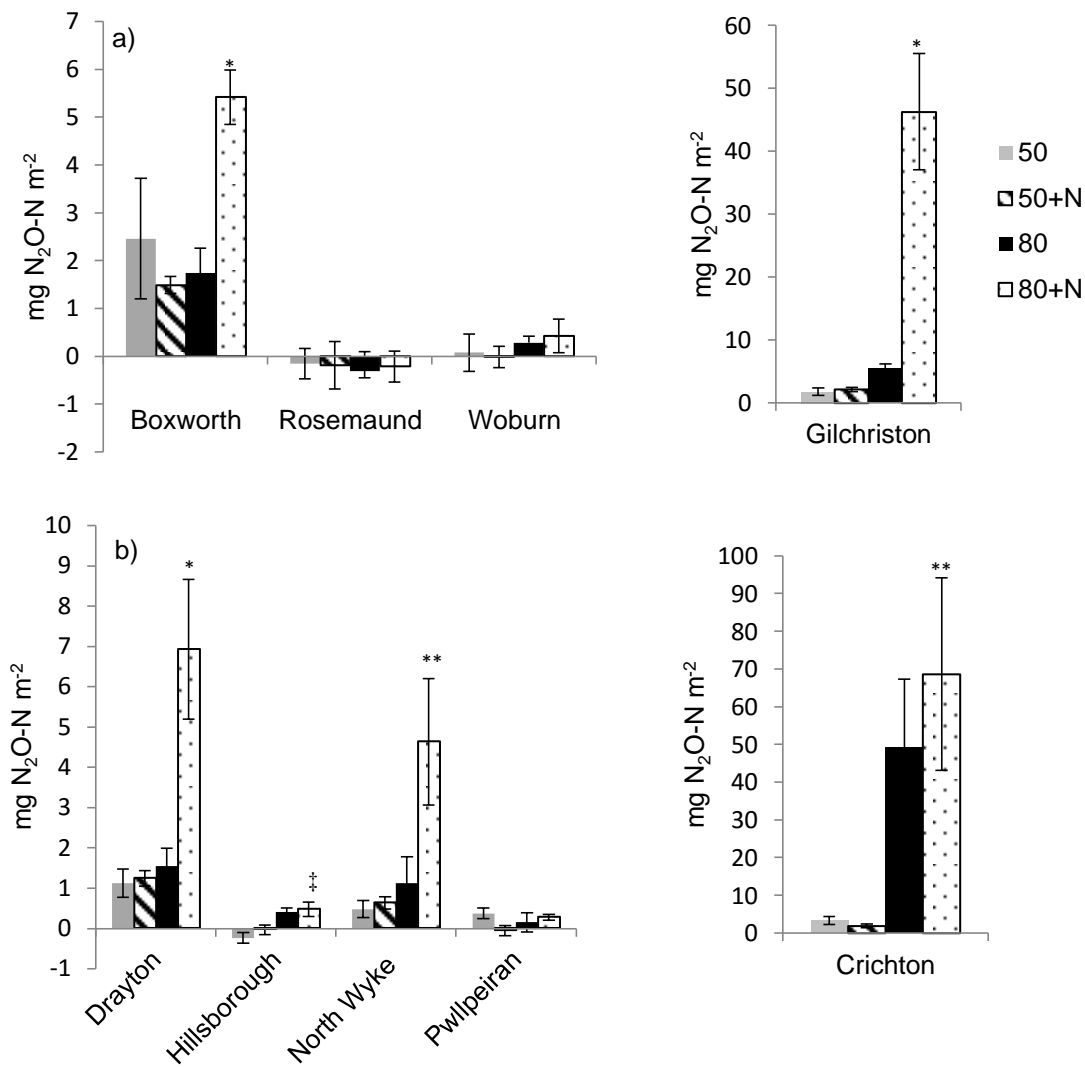


Figure 2.2: Cumulative N₂O fluxes (mg N₂O-N m⁻²) from a) arable and b) grassland soils for 50% water holding capacity (WHC) (50), fertilised 50% WHC (50+N), 80% WHC (80) and fertilised 80% WHC (80+N) treatments over a 22 day incubation period. Gilchriston and Crichton are plotted on separate scales for clarity. Two-way ANOVA, symbols denote significant difference from other treatments within the same soil, * p < 0.001, ** p < 0.005, † p < 0.05.

2.3.3 N₂O Emission Factors

The EFs calculated from the arable and grassland soils in this experiment (Figure 2.3) were consistently below the IPCC ‘default’ value of 1%. At 50% WHC the EFs were mostly negligible, ranging from (-0.02–0.01%). It is assumed that the moisture content is too low to allow for efficient nitrification (Maag and Vinther, 1996). EFs at 80% WHC ranged from 0.00–0.58%. Gilchriston had a significantly higher EF ($0.58 \pm 0.13\%$, $p < 0.01$) than all other soils at 80% WHC, except Crichton ($EF\ 0.28 \pm 0.15\%$).

The negative EFs do not indicate an uptake of N₂O from the atmosphere, but that the emissions from the unfertilised control treatments were greater than from the fertilised treatments. There were no significant differences between emissions from fertilised and unfertilised controls in cases where negative EFs were observed (Figure 2.3).

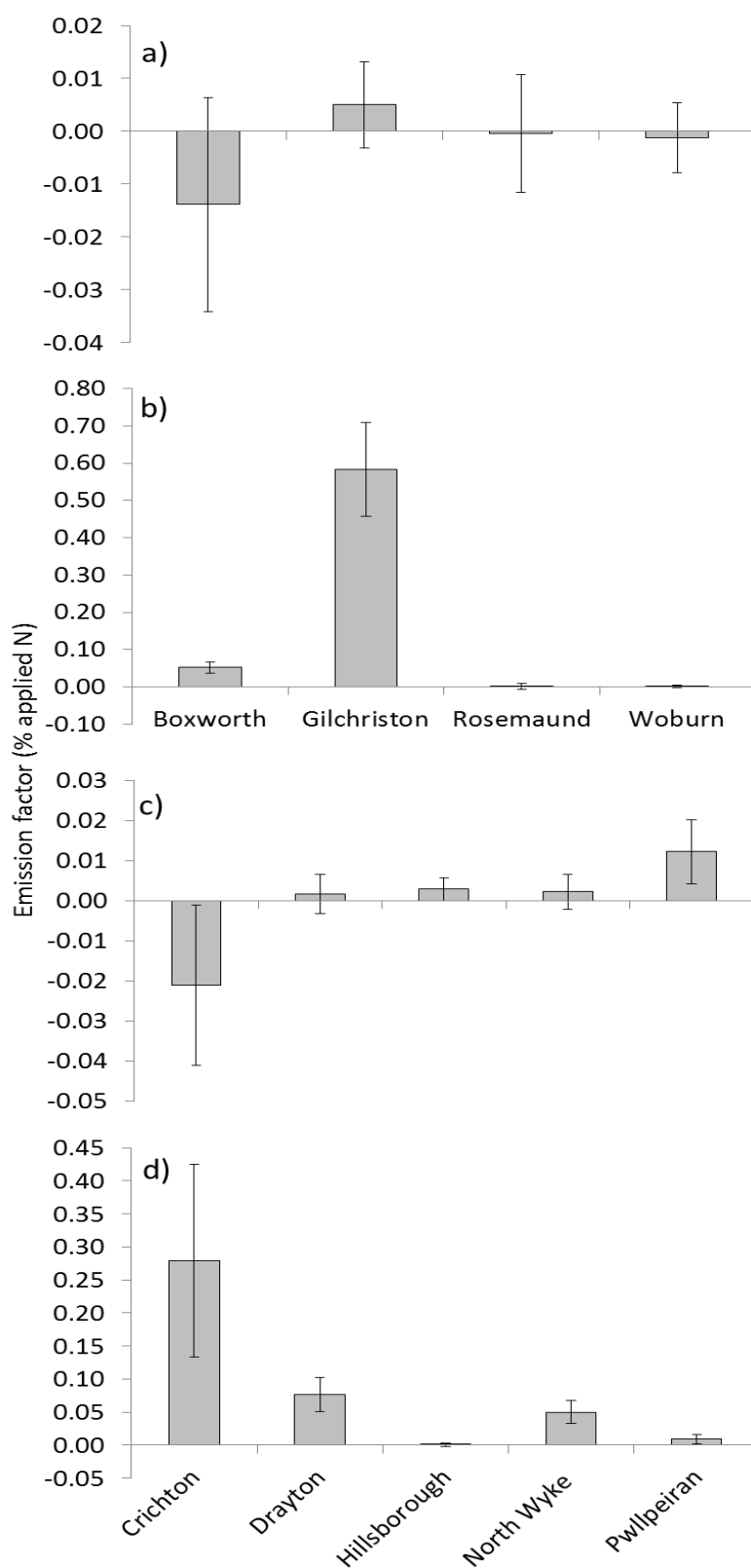


Figure 2.3: Emission factors for N_2O emissions from a) arable 50% WHC (water holding capacity), b) arable 80% WHC, c) grassland 50% WHC and d) grassland 80% WHC soils to which ammonium nitrate fertiliser had been applied at a rate of 70 kg N ha^{-1} .

2.3.4 CO₂ fluxes

There were no significant differences in cumulative CO₂ fluxes between treatments in the arable soils (Figure 2.4) and there was high variability between replicates. There was a slight interaction effect between moisture level and fertilisation for Woburn ($p < 0.05$, two-way ANOVA). In general there was an increase in CO₂ fluxes at 50% WHC when fertiliser was added and a suppression of fluxes at 80% WHC, except for the Boxworth soil where there was an increase.

There were also generally no significant differences in cumulative CO₂ fluxes between treatments in the grassland soils (Figure 2.4), although there was a significant effect of moisture content for Hillsborough and Pwllpeiran (two-way ANOVA, $p < 0.05$). Cumulative CO₂ fluxes generally tended to increase with addition of fertiliser at 50% WHC and for Hillsborough and Pwllpeiran at 80% WHC, although it was suppressed at 80% WHC for the other soils.

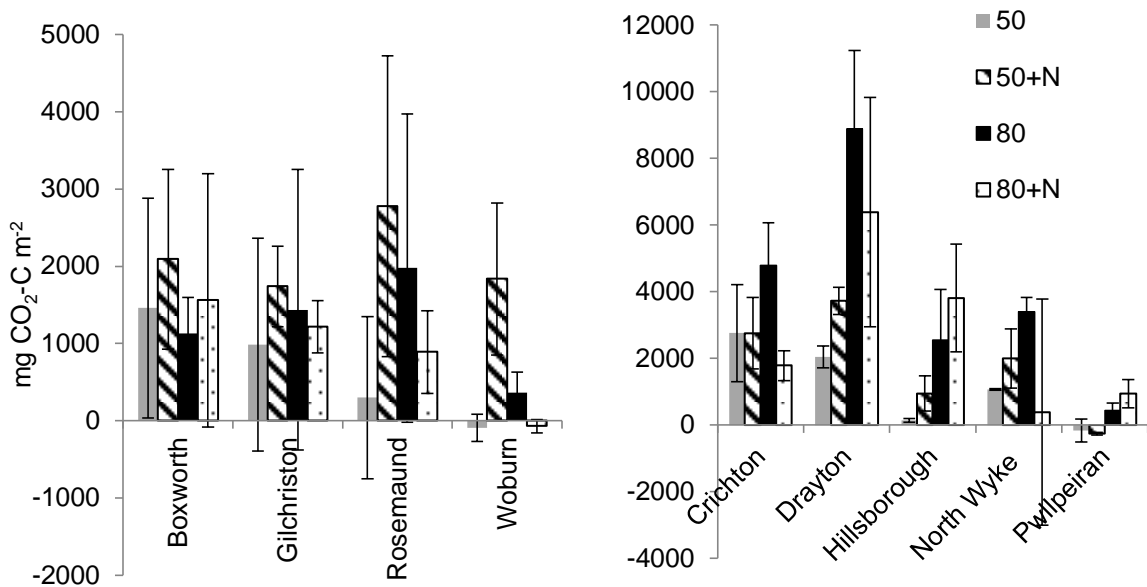


Figure 2.4: Cumulative CO₂ fluxes (mg CO₂-C m⁻²) for a) arable and b) grassland soils for 50% water holding capacity (WHC) (50), fertilised 50% WHC (50+N), 80% WHC (80) and fertilised 80% WHC (80+N) treatments over a 22 day incubation period (23 for Crichton).

2.3.5 CH₄ fluxes

The CH₄ fluxes calculated for each sampling day (Appendix 1) provided evidence that both methanogenesis and methanotrophy were occurring simultaneously in all soils with some alternating strongly between source and sink. The changes were much more pronounced for some soils than others. Calculated cumulative fluxes can be assumed to reflect the dominant process in each soil and treatment combination.

Cumulative CH₄ fluxes were highly variable and there were generally no significant differences in CH₄ fluxes between treatments within arable soils (Figure 2.5), except for the Rosemaund soil where there were significant increases due to the application of AN: from 0.44 ± 0.63 to 1.15 ± 0.08 mg CH₄-C m⁻² for 50% WHC and from 0.19 ± 0.26 to 1.36 ± 0.39 mg CH₄-C m⁻² for 80% WHC ($p < 0.05$, two-way ANOVA). In general there was an increase (or a reduced sink) in cumulative CH₄ fluxes with the addition of AN, except for Woburn where there was a reduction in cumulative CH₄ flux from 0.21 ± 0.77 to 0.01 ± 0.37 mg CH₄-C m⁻² for the 50+N treatment.

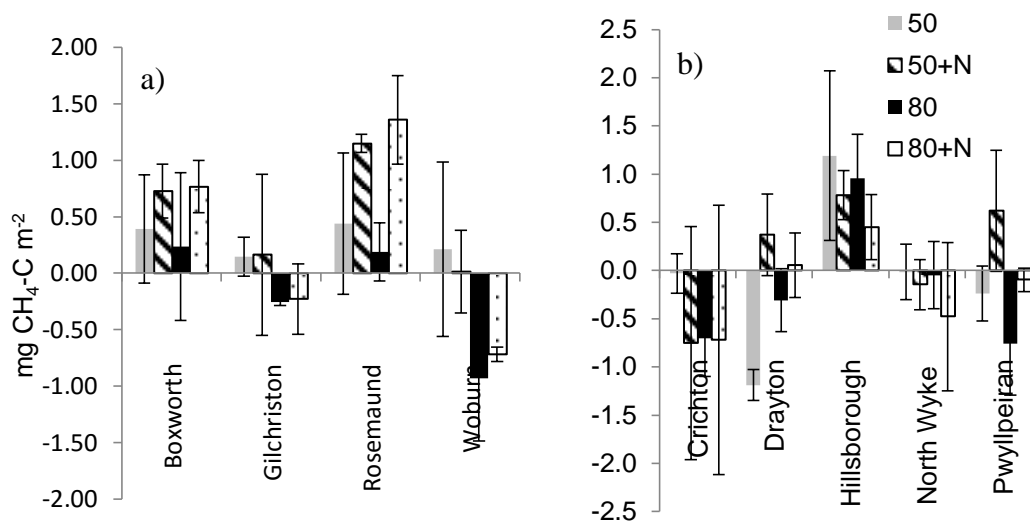


Figure 2.5: Cumulative CH₄ fluxes (mg CH₄-C m⁻²) for a) arable and b) grassland soils for 50% water holding capacity (WHC) (50), fertilised 50% WHC (50+N), 80% WHC (80) and fertilised 80% WHC (80+N) treatments over a 22 day incubation period (23 for Crichton).

There was also generally no significant effect of treatments on cumulative CH₄ fluxes from grassland soils (Figure 2.5). The exception being the Drayton soil which showed significant increases ($p < 0.05$, two-way ANOVA) in CH₄ flux with the application of AN, converting from a net sink to a source: from -1.19 ± 0.16 to 0.37 ± 0.42 mg CH₄-C m⁻² at 50% WHC and from -0.31 ± 0.32 to 0.05 ± 0.33 mg CH₄-C m⁻² at 80% WHC. It was not possible to identify a general trend across these grassland soils; at 50 and 80% WHC for Drayton and Pwllpeiran, and 50% WHC for North Wyke fertilisation increased CH₄ emissions (or decreased the sink), but decreased the emissions (or increased the sink) at 50 and 80% WHC for Crichton and Hillsborough and at 80% WHC for North Wyke. Increasing the moisture

content without AN application tended to decrease CH₄ emissions (i.e. increase sink strength).

2.3.6 Characterisation of Soils

The chemical characteristics of the soils were determined by FT-IR to determine if differences in CO₂ fluxes between soils could be correlated to differences in chemical structure. In whole soil samples it is easier to distinguish differences in soil mineralogy than OM composition due to overlapping absorption bands for several functional groups.

FT-IR spectra for the arable and grassland soils are shown in Figures 2.6 and 2.7, respectively. The greatest differences are at the 3400 and 3600 cm⁻¹ absorption bands (attributed to H bonded OH groups of OM and OH groups on mineral (e.g. kaolinite) surfaces). Soils with higher clay contents (Drayton, North Wyke and Boxworth) have sharp peaks at 3600 cm⁻¹, indicating the dominance of kaolinite OH groups, rather than other mineral or organic OH groups in these soils. The Crichton soil has low clay content and so the broad absorption band at 3400 cm⁻¹ is likely to be dominated by organic OH groups. The absorption band around 3400 cm⁻¹ is weaker for the Gilchriston soil compared with other arable soils.

The aliphatic C-H stretching groups (2920 and 2850 cm⁻¹) are most prominent in the Crichton, Pwllpeiran and Woburn soils.

The 1610 cm⁻¹ absorption band is indicative of aliphatic carboxylate groups, N-H deformation, C-N stretching and/or aromatic C=C groups are slightly weaker for North Wyke, Gilchriston and Woburn.

Strong absorption bands around 1000 cm⁻¹ indicate the dominance of Si-O stretches but C-O stretching of polysaccharide or polysaccharide-like substances also occur in this region. All grassland soils and the Rosemaund arable soil have particularly strong absorption bands in this region and Boxworth the weakest. The Hillsborough soil absorption band around 1000 cm⁻¹ is also slightly weaker than the other grassland soils, but this may be due to a poorer resolution spectrum being obtained for that soil sample.

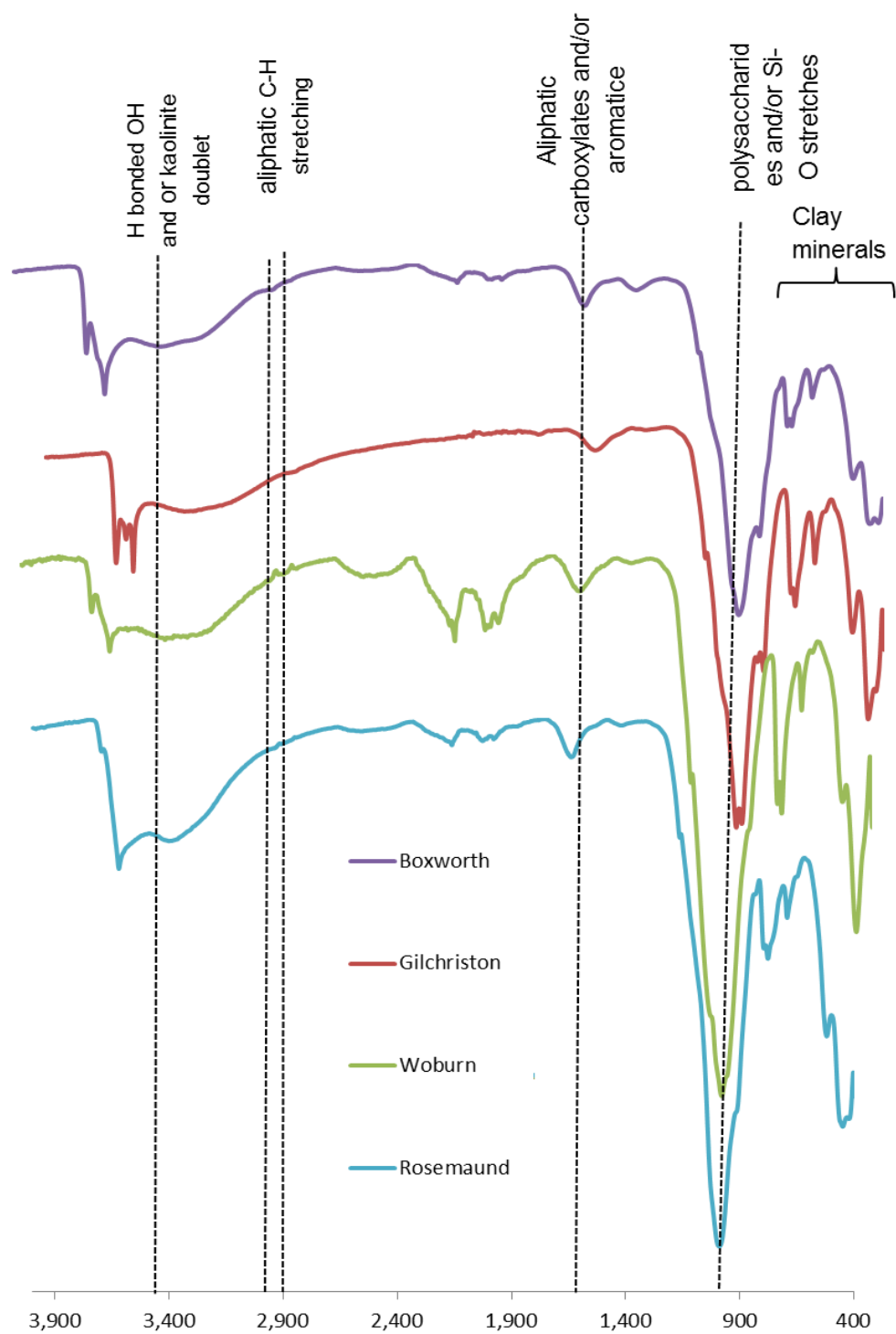


Figure 2.6: FT-IR spectra and assignments for 0–10 cm arable whole soils.

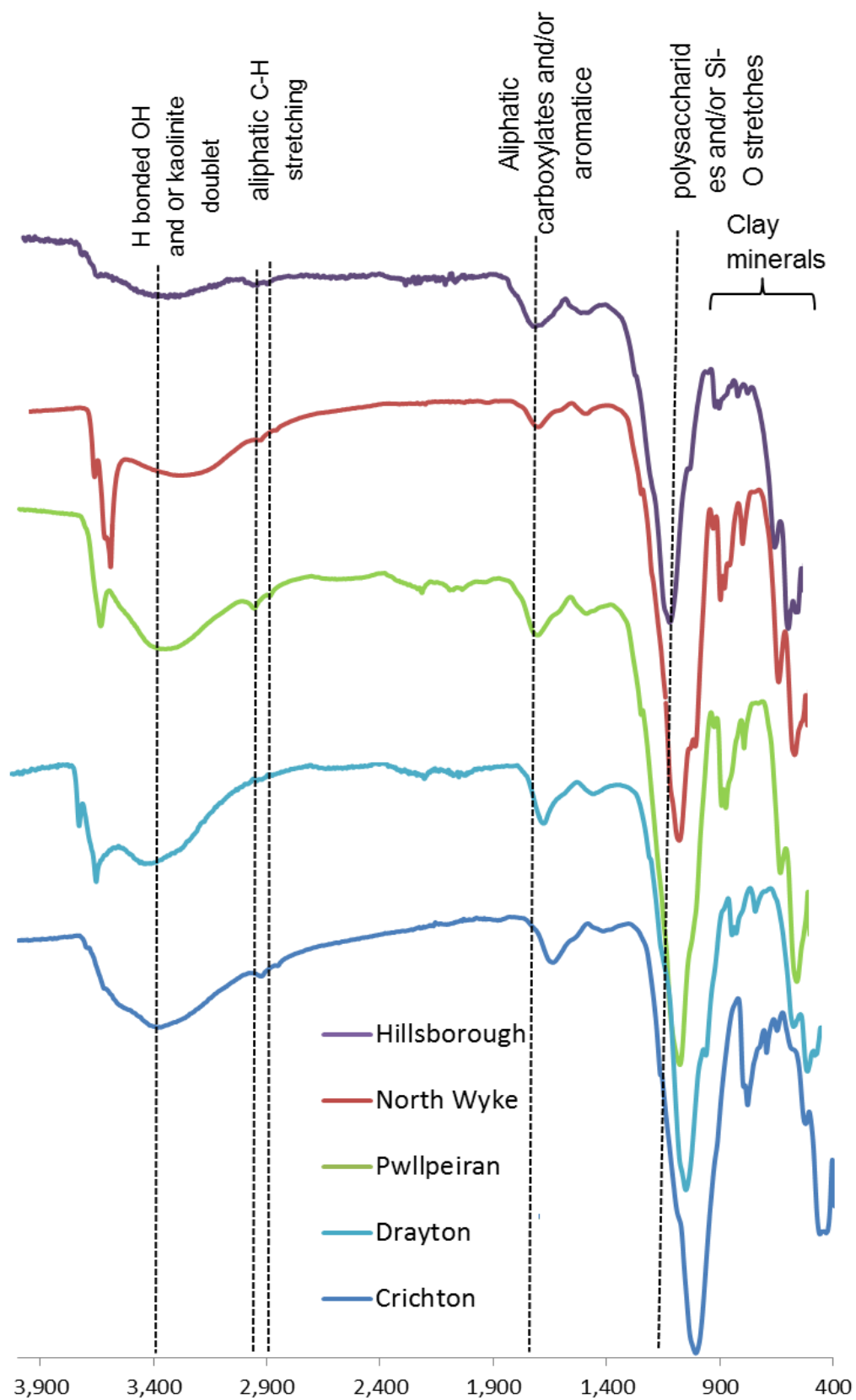


Figure 2.7: FT-IR spectra and assignments for 0–10 cm grassland whole soils.

2.3.7 Mineral N concentrations

For all soils there were large differences between initial untreated pre-incubation soil NO_3^- -N contents, but not corresponding NH_4^+ -N contents (Figure 2.8). Unfertilised and AN fertilised post-incubation Hillsborough and North Wyke grassland soils exhibited greatest loss or microbial transformation of native soil NO_3^- -N and added fertiliser NO_3^- -N. All AN fertilised post-incubation soils exhibited microbial transformation of added NH_4^+ -N but it was greatest for the Gilchriston arable soil. Results for the unfertilised post-incubation Crichton grassland soils suggest net production of NH_4^+ -N via OM mineralisation.

Moisture content had a small effect on the rate of NO_3^- -N mineralisation for grassland soils ($p = 0.04$) but not arable soils and no significant effect on NH_4^+ -N mineralisation. As expected, AN fertilisation had a significant effect on NO_3^- -N and NH_4^+ -N contents for both grassland and arable soils ($p < 0.001$) with increases (relative to unfertilised soils) between 5 and 25 times (for 50 and 80% WHC respectively) being observed.

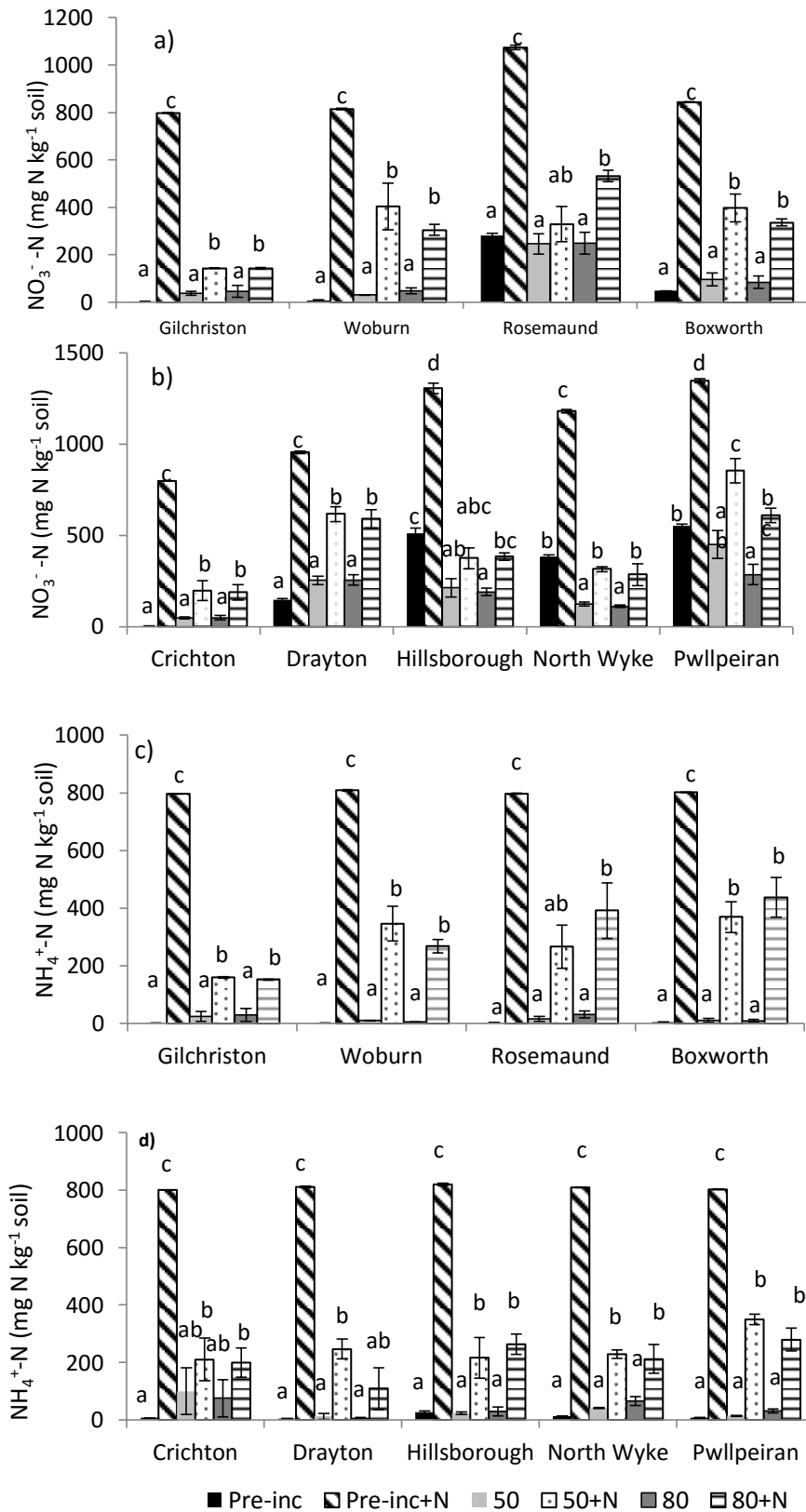


Figure 2.8: a) Arable soil NO₃⁻-N, b) grassland soil NO₃⁻-N, c) arable soil NH₄⁺-N and d) grassland soil NH₄⁺-N contents for untreated pre-incubation (pre-inc) soils and 50% WHC (50), fertilised 50% WHC (50+N), 80% WHC (80) and fertilised 80% WHC (80+N) post-incubation soils. Mean of three replicates ± standard error. Bars with different letters are significantly different (One-way ANOVA).

2.3.8 pH

Unfertilised Crichton samples had significantly lower pH ($p < 0.05$) at the end of the incubation than fertilised (Table 2.3). Conversely, fertilised Boxworth samples had significantly lower pH ($p < 0.01$) at the end of the incubation than unfertilised. All North Wyke and Rosemaund samples had significantly higher pH post incubation. For Woburn all samples had lower pH ($p < 0.01$) post incubation.

Table 2.3: pH for untreated and fertilised arable and grassland soils pre-incubation at 50% and 80% WHC, and control and ammonium nitrate (AN) fertilised arable and grassland soils post-incubation at 50 and 80% WHC. H^+ concentrations for individual soil treatments were used to analyse the data using a two-way ANOVA, except where the residuals were not normally distributed, in which case the log transformed data was used. * denotes significant difference from the pre-incubation pH ($p < 0.05$).

			50% WHC		80% WHC	
	Site	AN	Pre-incubation	Post-incubation	Pre-incubation	Post-incubation
Arable	Gilchriston	N	5.9	5.7 (0.3)	5.9	5.9 (0.1)
		Y	5.9	5.8	5.9	6.1
	Woburn	N	7.0	6.8	7.0	6.6 *
		Y	7.0	6.5 *	7.0	6.4 *
	Rosemaund	N	5.1	5.4	5.1	5.6 (0.2)
		Y	5.1	5.6 (0.2)	5.1	5.3
	Boxworth	N	7.9	7.9	7.9	7.9
		Y	7.9	7.6	7.9	7.6
Grassland	Crichton	N	5.3	4.8 (0.5)	5.3	5.1 (0.1)
		Y	5.3	4.9 (0.4)	5.3	5.2 (0.1)
	Drayton	N	6.4 (0.4)	6.7 (0.1)	6.4 (0.3)	6.7 (0.1)
		Y	6.8 (0.1)	6.7	6.7 (0.1)	6.5 (0.2)
	Hillsborough	N	5.9	5.7 (0.2)	5.9	5.6 (0.2)
		Y	5.9	5.6 (0.2)	5.9	5.7 (0.2)
	North Wyke	N	4.7	4.9 (0.1)	4.7	4.9 (0.1) *
		Y	4.7	4.9 (0.1) *	4.7	5.0 (0.1) *
	Pwllpeiran	N	5.1	4.9 (0.1)	5.1	4.9 (0.1)
		Y	5.1	5.0 (0.1)	5.1	5.1 (0.1)

2.3.9 Bivariate Correlations

Bivariate correlations between measured soil properties for the nine Defra soils are presented in Table 2.4: Soil NO_3^- -N contents were positively correlated with OC, TN and NH_4^+ -N contents and negatively correlated with soil pH. TN and pH were positively correlated with clay content and C:N was negatively correlated with clay content. C:N was correlated positively with pH and unsurprisingly C:N, OC and TN contents were all positively correlated to each other.

Table 2.4: Bivariate correlations between measured soil properties (pre-treatment) for all nine soils. Clay, total carbon (OC), total nitrogen (ON) NH_4^+ and NO_3^- contents, pH and C:N. * Significant correlations ($p < 0.05$).

Variate	Clay (% by weight)	OC (% by weight)	TN (% by weight)	C:N	pH	NH_4^+ -N (mg kg ⁻¹)	NO_3^- -N (mg kg ⁻¹)
clay	1.00						
OC	0.19	1.00					
TN	0.33 *	0.96 *	1.00				
C:N	-0.21 *	0.69 *	0.51 *	1.00			
pH	0.26 *	-0.06	-0.16	0.27 *	1.00		
NH_4 -N	0.00	0.02	0.01	0.01	0.02	1.00	
NO_3 -N	0.09	0.27 *	0.31 *	0.00	-0.23 *	0.89 *	1.00

2.3.10 General Linear Modelling

Results from both full and reduced models are shown for arable (Table 2.5) and grassland (Table 2.6) soils. Some outliers were removed from the analysis after scrutiny of the residuals. These were:

1. Grassland CO_2 analysis: Drayton, block 3, 80+N.
2. Grassland CO_2 analysis: North Wyke, block 1, 80+N.
3. Grassland CH_4 analysis: Crichton, block 3, 80.
4. Arable CH_4 analysis: Woburn, block 3, 50.

Table 2.5: Full and reduced multiple linear regression models for arable soils. Variables assessed were NO₃-N, NH₄-N, % water holding capacity (WHC), total carbon (OC % by weight), total nitrogen (TN % by weight), C:N, clay (% by weight) and pH. CV is the coefficient of variance, SE is the standard error. Significance levels are denoted as follows: * p < 0.001, ** p < 0.005, *** p < 0.01, **** p < 0.05.

Variable	Arable CO ₂				Arable N ₂ O				Arable CH ₄			
	Full Model		Reduced Model		Full Model		Reduced Model		Full Model		Reduced Model	
	CV	SE	CV	SE	CV	SE	CV	SE	CV	SE	CV	SE
NO ₃ ⁻ -N	-1732	4.33			0.00048	0.000671	0.0003 ****	0.0000				
NH ₄ ⁺ -N	-2.43	4.36			-0.000178	0.000684			-0.0003	0.0002		
% WHC	19	15.4			0.0061	0.0032			0.0146 ****	0.0065	0.0146 ****	0.0066
OC	3024	2535	1082	604	2.798 *	0.486	2.723 *	0.328				
TN	-19479	23840							94.4 **	28.2	72.9 *	18.5
C:N									0.0851	0.0827		
clay					-0.0658 *	0.013	-0.0638 *	0.0089	-0.243 **	0.078	-0.1855 *	0.0535
pH												
Constant	-1732	1458	-392	818	-2.361 *	0.454	-1.774	0.259	-7.73 **	2.56	-5.59 *	1.2
F	p = 0.328		p = 0.08		*		*		*		*	
R ²	0.02		0.05		0.61		0.60		0.39		0.37	

Table 2.6: Full and reduced multiple linear regression models for grassland soils. Variables assessed were NO₃-N, NH₄-N, % water holding capacity (WHC), organic carbon (OC % by weight), total nitrogen (TN % by weight), C:N, clay (% by weight) and pH. CV is the coefficient of variance, SE is the standard error. Significance levels are denoted as follows: * p < 0.001, ** p < 0.005, *** p < 0.01, **** p < 0.05.

Variable	Grassland CO ₂				Grassland N ₂ O				Grassland CH ₄			
	Full Model		Reduced Model		Full Model		Reduced Model		Full Model		Reduced Model	
	CV	SE	CV	SE	CV	SE	CV	SE	CV	SE	CV	SE
NO ₃ ⁻ -N	7.32	8.73	-6.26 *	1.24	-0.0022 **	0.0007	-0.0018 *	0.0002	0.0030	0.0035		
NH ₄ ⁺ -N	-4.46	8.86	9.32 *	1.41	0.0028 *	0.0007	0.0023 *	0.0002	-0.0033	0.0035		
% WHC	8.8	17.1			0.003	0.0024			0.0120	0.0067		
OC					-0.094	0.114			0.008	0.437	0.1036 *	0.0279
TN	-39464	24582			2.26	3			-8.08	8.96		
C:N	2108	1239							0.549	0.608		
clay	113.9 *	33.6	88.7 *	19.5	-0.00537	0.00427	-0.008 **	0.0028				
pH	2127	1458			-0.206	0.206			0.253	0.564		
Constant	-22114	12672	526	818	1.466	0.608	0.7506*	0.0806	-5.36	5.99	-0.509 **	0.184
F	*		*		*		*		***		*	
R ²	0.54		0.52		0.70		0.69		0.20		0.18	

For all of the model runs, except for the arable cumulative CO₂ flux, the full model explained 1–2% more of the variation than the reduced model, which contained only variables which explained a significant amount of variation.

It was not possible to adequately describe the variation of cumulative CO₂ fluxes from the arable soils with the measured variables (Table 2.5). CO₂ fluxes from arable soils were much more variable than from grassland soils (see Appendix 1). The full model described only 2% of the variation. No variate, or combination of variates, were significant, however OC contents described the largest amount of variation (5%, $p = 0.08$). For arable cumulative N₂O fluxes, the reduced model explained 60% of the variation with significant positive correlations with OC ($p < 0.001$) and NO₃⁻-N ($p < 0.05$) contents, and negative correlation with clay contents ($p < 0.001$). The reduced model for arable cumulative CH₄ fluxes explained 37% of the variation with significantly positive correlations with TN contents ($p < 0.001$) and % WHC ($p < 0.05$), and negative correlation with clay contents ($p < 0.001$).

The reduced models explained 52% of the variation for cumulative CO₂ fluxes from the grassland soils (Table 2.6). Cumulative CO₂ fluxes had significant ($p < 0.001$) positive correlations with NH₄⁺-N and clay contents and significant negative correlation with NO₃⁻-N contents. For grassland cumulative N₂O fluxes the reduced model explained 69% of the variation with significant negative correlations with NO₃⁻-N ($p < 0.001$) and clay ($p < 0.005$) contents and positive correlation with NH₄⁺-N ($p < 0.001$) contents. The reduced model for grassland cumulative CH₄ fluxes explained 18% of the variation with a significant positive correlation with OC contents ($p < 0.001$).

It appears that the most important explanatory variables were NH₄⁺-N, NO₃⁻-N, clay and OC contents. The exception was the arable cumulative CH₄ fluxes where TN contents and % WHC (as well as clay contents) were significant explanatory variables.

2.4 Discussion

2.4.1 CO₂ fluxes from UK arable and grassland agricultural soils

Carbon dioxide fluxes from the arable soils were highly variable and as a result the multiple regression model was unable to explain the variation in any meaningful way. The C concentrations of arable soils did explain 5% of the variation and was positively correlated to CO₂ flux; this is presumably related to the amount of C available for decomposition by the microbial biomass. Other studies have found that soil CO₂ flux is positively correlated with soil C concentration: Franzluebbers *et al.*, (1995) found that CO₂ flux increased with OC concentration from conventionally tilled arable soils in a long term field experiment; Wang *et al.* (2003) found that SOC concentration explained 75–81% of the variation in CO₂ flux in a wide range of incubated Australian soils.

The significant explanatory variables for cumulative CO₂ fluxes from the grassland soils were clay and mineral N concentrations. A positive correlation between CO₂ fluxes and clay content is counter to the theory that higher clay contents provide a greater opportunity for chemical protection of OM by adsorption. Dilustro *et al* (2005) also observed greater CO₂ fluxes from clayey textured (> 19% clay) than sandy textured (< 12% clay) forest soils. However, they attribute this to a more dense vegetation (and so greater root respiration) on the clayey soils and the sandy soils being excessively drained for part of the study period. A negative correlation between clay content and CO₂ flux was expected and has been found by Wang *et al* (2003) and Bauer *et al* (2006) for arable soils. However, no effect of soil texture has also been reported by Franzluebbers (1999) and Bouma and Bryla (2000).

Grassland soil CO₂ flux showed a positive correlation with NH₄⁺-N concentration and a negative correlation with NO₃⁻ concentration. Reduced CO₂ flux has been recorded for a forest soil fertilised with an NO₃ based fertiliser (Burton *et al*, 2004), consistent with this study. A decrease in the microbial biomass was observed at this site which may explain the lowered soil CO₂ flux (DeForest *et al*, 2004). Conversely, soil fertilised with NH₄ based fertiliser had no effect on microbial biomass in a study carried out by Zaman *et al* (2002). Zaman *et al* (2002) speculate that fertilisation without the addition of C cannot drive increased microbial growth or respiration. In this study fertilisation with AN was observed to decrease (mostly at 80% WHC) and have no effect on CO₂ emissions (mostly at 50% WHC). There are several conflicting results in the literature which show increases (Baggs *et al*, 2003), decreases (al-Kaisi *et al*, 2008) and no effect (Baggs *et al*, 2003; Garcia-Ruiz and Baggs, 2007 and al-Kaisi *et al*, 2008) of AN fertilisation on CO₂ flux.

Despite there being some differences in the FT-IR spectra of the whole soils, particularly between the grassland soils, it has been difficult to relate this to the differences in CO₂ fluxes. For example, the Crichton soil has relatively stronger absorption bands at 1000 cm⁻¹ and 3400 cm⁻¹ and a low clay content which would suggest that the soil contains more phenolic compounds and polysaccharides than the other grassland soils, but did not have significantly greater CO₂ emissions.

North Wyke, Pwllpeiran and Rosemaund had low C:N and similar FT-IR spectra, North Wyke and Pwllpeiran had lower cumulative CO₂ fluxes than other grassland soils but Rosemaund did not have lower cumulative CO₂ fluxes than the other arable soils.

2.4.2 CH₄ fluxes from UK arable and grassland agricultural soils

Individual soils showed highly variable CH₄ fluxes throughout the incubation period and evidence that both methanogenesis and methanotrophy were occurring simultaneously (Ekberg and Christensen, 2006).

Soil moisture content has been identified as the main driver of CH₄ flux as methanogenesis requires anaerobic soil conditions and specific redox conditions, and methanotrophy requires aerated soils to facilitate diffusion of CH₄ into the soil from the atmosphere (Hiltbrunner *et al*, 2012; Cloy and Smith, 2015). In this study soil moisture did not have a significant effect on any individual soil, and increasing the moisture content from 50 to 80% WHC actually reduced emissions (increased sinks) in all cases except for the Drayton soil. The Drayton 80+N treatment was interesting because it showed high CH₄ emissions, high N₂O emissions and low CO₂ emissions, which would suggest that there were anaerobic micro-sites present during incubation of this clay soil.

It is possible that the methanotrophic microbial population was under water stress at the lower WHC level. von Fischer *et al* (2009) found that methanotrophic activity dropped off sharply below 40% WFPS in a sandy loam grassland soil. Despite this, % WHC did explain a small (2.8%) but significant ($p < 0.05$) amount of the variation in the arable soils.

Inorganic fertiliser application did not explain a significant amount of variation in CH₄ fluxes in this study. However, when looking within individual soils, increased emissions (Rosemaund), conversion from sink to source (Drayton and Pwllpeiran), decreased emissions (the 50+N treatments for Woburn, Crichton and Hillsborough, and the 80+N treatment for Crichton, Hillsborough and North Wyke although these were insignificant) and no effect on CH₄ flux were observed.

Studies have reported inhibition of methanotrophs, leading to increased CH₄ emissions (or reduced sink strength) in forest soils (Jassal *et al*, 2011) arable soils (Acton and Baggs, 2011) and grassland soils (Baggs and Blum, 2004) after the application of inorganic N fertiliser, particularly ammonium based fertilisers such as AN. This reduction in CH₄ uptake is often accompanied by an increase in N₂O emissions and is attributed to competition between NH₃ and CH₄ for CH₄ monooxygenase enzymes (Acton and Baggs, 2011). However, the only discernible increase in N₂O emissions alongside increased CH₄ emissions was for the Drayton 80+N treatment.

Another mechanism suggested for the inhibition of methanotrophs is the toxicity of NO₃⁻, which is produced during the breakdown of NH₃, on methanotrophic microbes, but only when atmospheric concentrations of CH₄ are low (King and Schnell, 1994).

Decreased CH₄ emissions after inorganic N fertilisation have been less widely reported but Hellebrand *et al* (2003) found a 4–9% and 24–62% increased uptake of CH₄ by a sandy loam arable soil after 75 and 150 kg N ha⁻¹ NPK fertiliser application, respectively, over a 3 year period. It was suggested that increased growth of methanotrophs and/or suppression of methanogens due to fertilisation was responsible for reducing the CH₄ concentration in the

soil and so causing an enhanced diffusion into the soil from the atmosphere (Hellebrand *et al.*, 2003).

Bodellier *et al* (2000) found that ammonium based fertilisers do not necessarily result in inhibited CH₄ oxidation, but that if there is a high CH₄ availability, then competition from ammonia would be 'counterbalanced'.

The most important explanatory variables for cumulative CH₄ fluxes from arable soils were the native (i.e. pre-treatment) N content and clay content of the soils. Soils with coarse textures (lower clay and higher sand contents) have higher uptake of atmospheric CH₄ than more fine textured soils, attributable to low porosity and high water retention in fine textured soils causing low gas diffusivity into the soil (Dörr *et al*, 1993; Dutaur and Verchot, 2007; Tate *et al*, 2007). This is in agreement with the results for the arable soils in this study; Gilchriston and Woburn had lower clay contents than Rosemaund and Boxworth, lower emissions at 50% WHC and were net sinks at 80% WHC whereas both Rosemaund and Boxworth were both net sources for all treatments (Figure 2.5).

The native N concentration explained 12.9% of the variation in CH₄ fluxes for arable soils. The N concentration of all soils was positively correlated to clay content (see Table 2.4). Rosemaund and Boxworth also had higher native N concentrations than Gilchriston and Crichton.

The only variable that explained a significant amount of CH₄ flux variation from the grassland soils was the soil OC concentration. Weslien *et al* (2009) also found a positive correlation between CH₄ and soil C concentration. However, when fertilised and unfertilised treatments are treated separately, NH₄⁺-N is positively correlated with CH₄ flux ($R^2 = 0.83$ for unfertilised and $R^2 = 0.46$ for fertilised soil, see Figure 2.9) and becomes the only significant explanatory variable in the multiple-regression models ($R^2 = 0.17$ for unfertilised and 0.19 for fertilised).

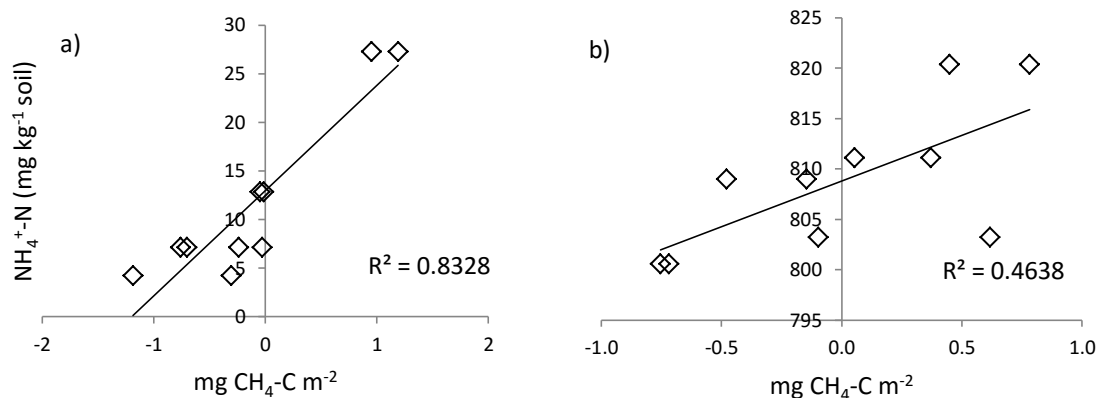


Figure 2.9: Linear regressions for grassland soil CH_4 fluxes (mg $\text{CH}_4\text{-C m}^{-2}$) against soil $\text{NH}_4^+\text{-N}$ (mg kg^{-1} soil) a) without and b) with ammonium nitrate fertilisation at 70 kg N ha^{-1} .

2.4.3 N_2O fluxes from UK arable and grassland agricultural soils

Clay content was found to be an important factor in determining N_2O fluxes from both arable and grassland soils in this study. Stehfest and Bouwmann (2006) conducted a meta-analysis of N_2O measurements from over 1000 agricultural sites and found that SOC content, pH and soil texture controlled N_2O flux. They explained the influence of soil texture on N_2O fluxes through its impact on soil water dynamics. Fine textured soils have smaller pores and so anaerobic microsites are more likely to develop within the soil structure leading to higher N_2O fluxes. However, in this study N_2O flux was negatively correlated to clay content. Crichton and Gilchriston in particular had lower clay contents but considerably higher N_2O emissions. However, this seems to be atypical and when they are removed from the analysis the relationship becomes positive and stronger (Figure 2.10).

Dobbie and Smith (2003) found that WFPS, temperature and $\text{NO}_3^- \text{- N}$ content were the most important factors affecting UK agricultural soils.

The two Scottish soils display substantially higher (up to ~ 140 and ~ 50 times higher for Gilchriston and Crichton, respectively) N_2O fluxes than the other soils, particularly for the $80+\text{N}$ treatment. A week after the application of AN (at a rate of 80 kg ha^{-1}), N_2O fluxes of approximately $8 \text{ mg N}_2\text{O-N m}^{-2} \text{ d}^{-1}$ were found at Gilchriston, soil WFPS was typically lower than 50% (Winning, 2014; Hinton *et al*, 2015). Bell *et al* (2015a) then compared annual cumulative N_2O fluxes measured in the field at Gilchriston, Rosemaund and Woburn sites after AN fertiliser application. Gilchriston fluxes were ~ 2.0 and ~ 2.5 times greater than those measured at Woburn and Rosemaund, respectively. For the Crichton grassland site, Bell *et al* (2016) found annual cumulative N_2O fluxes of approximately $0.2 \text{ g N}_2\text{O-N m}^{-2}$

after 8 g m^{-2} AN application and soil WFPS ranged from 71 to 58% over the one-year measurement period.

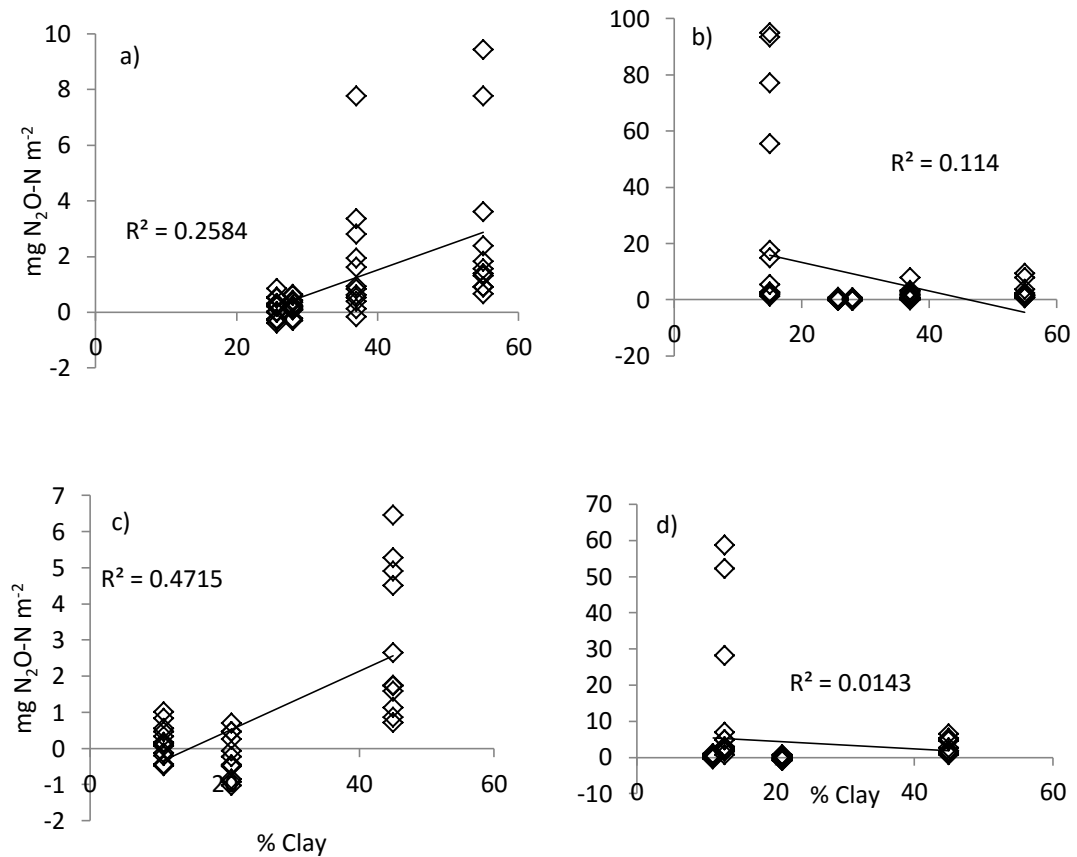


Figure 2.10: Cumulative $\text{N}_2\text{O-N}$ fluxes ($\text{mg N}_2\text{O-N m}^{-2}$) against % clay contents for grassland soils a) excluding or b) including Crichton, and for arable soils c) excluding or d) including Gilchriston.

Skiba and Ball (2002) also found that the clay content of soils is an important factor controlling N_2O fluxes. They measured fluxes along a transect across 13 fields and covering 10 different soil series. N_2O fluxes were measured in March before and after the application of AN to sub plots at a rate of 120 kg N ha^{-1} , then in May and October of the same year. They found that bulk density and clay content were the variables which best explained differences in fluxes along the transect, although this was only significant for the May flux measurements and was probably masked by the complex interactions between soil mineral N concentrations, moisture and temperature in the field (Skiba and Ball, 2002).

Nitrous oxide flux was found to have a positive correlation with SOC concentration by Stehfest and Bouwmann (2006) in their meta-analysis. This was generally true for this study; however Gilchriston, which had a lower SOC concentration (1.27%) than Boxworth (1.90%), had much higher N_2O fluxes, likely due to the presence of carbonates (~13% of total C) in the Boxworth soil. The amount of available C in soil can influence the rates of

nitrification and denitrification through provision of substrate for the microbial community involved (Pihlatie *et al*, 2004).

The moisture content of the soils did not explain a significant amount of the variation in N₂O fluxes, but did increase the rate of NO₃⁻-N mineralisation for the grassland soils. Franzluebbers (1999) found a sharp increase in NO₃⁻-N mineralisation with WFPS, up to a critical point above which it subsequently decreased. Many studies have found a positive correlation between N₂O flux and soil moisture content (Ciarlo *et al*, 2008). Pihlatie *et al* (2004) found that N₂O fluxes did not increase for WFPS between 40 and 80% for a peat and a clay soil but increased by 95 and 460 times, respectively at 100% WFPS. However, in a loamy sand soil in the same experiment, N₂O production increased logarithmically with increasing moisture content. This supports the results found here that dry soils have very low N₂O fluxes. As soil moisture increases, soil O₂ concentrations decrease and at moisture contents > 60% WFPS denitrification, rather than nitrification, becomes the dominant process for N₂O production (Ruser *et al*, 2006).

2.4.4 N₂O emission factors

In this study, EFs were consistently below the value assumed in the IPCC Tier 1 methodology (factor of 1%) (IPCC, 2006). The Gilchriston site had the highest EF at 80% WHC which had an EF around half of the default value (0.58%) . Hinton *et al* (2015) conducted a field experiment at the Gilchriston site where AN was applied at different rates and N₂O emissions were monitored for one year. They found EFs for the five weeks following AN application ranging from 0.44–0.56%, but annual EFs of 1.36, 0.96 and 1.08 for AN application rates of 120, 160 and 200 kg N ha⁻¹.

Smith *et al* (2012) monitored N₂O emissions from AN fertilised and control plots at the Crichton, Hillsborough and Boxworth sites for one growing season. They found an EF of 0.61% for Crichton in 2004, which is similar to the value found in this study under controlled conditions; however the EF in 2003 was higher (1.13%). EFs are highly variable, and although the IPCC recommend a default value of 1%, the range of uncertainty associated with this value is 0.3–3.0% (IPCC, 2006b). Dobbie and Smith (2003) monitored N₂O emissions from a representative range of agricultural soils from across the UK for two years and calculated annual EFs ranging from 0.4 to 6.5% for grassland and 0.5 to 1.5% for arable sites. EFs of between 0.2 and 7% have been reported for agricultural fields in Scotland (Clayton *et al*, 1997; Dobbie *et al*, 1999; Smith *et al*, 1998).

It has been suggested that the higher EFs from Scottish sites is due to the incidence and intensity of rainfall (and therefore WFPS) at the time of fertiliser application (Dobbie *et al*, 1999). However, this does not explain the higher EFs from Scottish arable and grassland

sites, compared to those elsewhere in the UK, under the controlled conditions in this experiment. However, it may be speculated that the high N₂O emissions are caused by differences in the microbial communities present in the Scottish soils driven by climatic factors (Castro *et al*, 2010). For example the wetter climate in Scotland may cause a difference in the fungal community abundance and structure (Castro *et al*, 2010). Fungi are known to increase denitrification (Shoun *et al*, 2012). However, Hillsborough in Northern Ireland would be expected to have similar climatic conditions to Scotland but did not display the same high N₂O flux when fertilised at higher moisture contents.

2.5 Limitations

Negative CO₂ fluxes were observed on some sampling days in all soil except for Drayton (see Appendix 1). There are four possible explanations for this phenomenon:

1. CO₂ concentration differences between t0 and t1 headspace gas samples were not large enough to accurately detect a concentration difference between them, indicating very low respiration and leading to a small negative flux being reported.
2. It is possible that not all Kilner jars were air tight during all closure periods. Although all jars were tested to ensure that they were air tight at the beginning of incubations and rubber seals were smeared with silicone based vacuum grease (Dow Corning, High Vacuum Grease, Dow Corning, Michigan, USA) it is possible that small amounts of air were able to escape as the pressure in the jars increased during closure periods.
3. Some of these soils contain carbonates and, under alkaline conditions, they can uptake atmospheric CO₂ causing carbonate dissolution to form Ca(HCO₃)₂ (Buysse *et al* 2013). For soils with pH > 7 incubated at 10% moisture content, Ma *et al* (2013) found negative CO₂ fluxes attributed to inorganic C dissolution and Buysse *et al* (2013) observed negative CO₂ fluxes during the cooling of incubated soil.

A further limitation of this study was that the soils had been air dried and stored so the microbial community developing in these soils might only be a subset of the microbial community structure in the field. The soils were also incubated after re-wetting without any pre-incubation to allow the microbial community to develop and stabilise. Re-wetting dry soil has been shown to de-couple soil C dynamics from the soil microbial community structure (Blazewicz *et al*, 2014; Barnard *et al*, 2015).

2.6 Conclusions

Soil texture, soil mineral N concentrations and SOC concentrations were found to be the most important measured variables controlling GHG fluxes from the UK agricultural soils used in this study. The results were generally in support of those found in the literature for a wide range of soils, conditions and locations; however, N₂O emissions from the two Scottish soils appeared to be more sensitive to AN fertilisation at 80% WHC than the other soils, with the N₂O emissions being exceptionally high in comparison.

Although incubation methods are a useful means of studying the impacts of agricultural land management practices on CO₂ (and other GHG) emissions from soil, they do not provide any

information about the mechanisms which stabilise SOC and how management practices impact on this. To study the physical and chemical mechanisms which control SOC stability it is necessary to physically separate the soil into pools stabilised by different mechanisms using soil fractionation.

3 Review of Fractionation Methods

Soil fractionation has been used widely to isolate soil organic matter (SOM) pools which are operationally defined, but functionally homogenous. Techniques used include size, density and chemical separation, described in detail below. In this section I present a short review of different techniques used in the literature and describe the three fractionation procedures used in the experimental work in this thesis.

3.1 Common Fractionation Techniques

3.1.1 *Dispersion of soil prior to, or during, fractionation*

Most fractionation methods require, at some stage, that the soil is dispersed (separated in to single particles). The two most commonly used methods of dispersing soil are by shaking (with beads or a dispersing agent) or by ultrasonic dispersion (sonication). Shaking soil in deionised water with glass or agate beads is a widely used method of breaking aggregates apart; however, calculating the energy input of this is understandably difficult and likely to be inaccurate.

Shaking soil with sodium based dispersing agents (e.g. sodium pyrophosphate or sodium hexametaphosphate) can completely disperse soil by replacing polyvalent cations with the monovalent Na^+ ion, preventing clay particles from flocculating (Elliott and Cambardella, 1991). Stemmer *et al* (1998) found that high energy sonication of soil gave a similar particle size distribution as shaking with sodium pyrophosphate, although significantly greater separation of clay sized particles from silt sized particles was obtained by the dispersing agent than by sonication.

Sonication is the process of subjecting a substance to high-frequency (> 20 kHz) sound waves, causing its particles to vibrate and cavitation to occur; when the bubbles formed on cavitation collapse they produce sufficient energy to break apart loosely held together aggregates (Elliott and Cambardella, 1991).

Sonication can lead to the transferral of OM to finer sized fractions (Elliott and Cambardella, 1991; Cambardella and Elliott, 1993; Kaiser and Guggenberger, 2007; Schrumpp *et al*, 2013). Kaiser and Guggenberger (2007) found that up to 100% of organo-mineral OM could be re-distributed by sonication, whilst only < 5% was redistributed when gently agitated in sodium polytungstate (SPT). However, a dispersion energy which is too low will result in occluded OM being allocated to the mineral bound fraction (Cerli *et al*, 2012). Stemmer *et al* (1998) compared the dispersion by shaking method of Monrozier *et al* (1991) with the sonication method and found that shaking redistributed a large proportion of the sand fraction to the silt sized fraction (63–2 μm

diameter) without increasing the clay fraction (2–0.1 μm diameter). They conclude that low energy sonication allows for the breaking apart of macro-aggregates and unstable micro-aggregates which could be disrupted by normal environmental factors (ploughing, rainfall, etc.), whilst leaving micro-aggregates in the silt sized fraction intact. Amelung and Zech (1999) investigated sonication at different output energies to assess the level of transferral of C and N between different particle size fractions. Their findings suggested that POM should be removed prior to any dispersion with an energy input greater than 5 kJ, and that a subsequent dispersal at ≥ 22 kJ would disrupt aggregates $< 250 \mu\text{m}$ without creating C concentration artefacts.

3.1.2 Particle-size fractionation

Partitioning soil by particle size allows an analysis of the aggregate size hierarchy (Gerzabeck *et al*, 2006). Methods range from a very simple two pool method (Cambardella and Elliott, 1992; Adewopo *et al*, 2015) to more intensive methods involving a combination of wet and repeated sedimentation to yield six different size fractions (Monrozier *et al*, 1991). Monrozier *et al* (1991) found some redistribution of microbial biomass C to the smallest sized fractions caused by disruption of the macro-aggregates (shaking with beads).

Wet sieving can cause slaking of aggregates, particularly if the soil is dry prior to sieving, and leading to a redistribution of larger aggregates to smaller size pools (Elliott and Cambardella, 1991; Van Hemelryck *et al*, 2010).

3.1.3 Density separation

Density fractionation is the separation of light, recently incorporated SOM from more microbially processed, mineral bound SOM in a density separation solution and is frequently used alongside particle fractionation. SOM lowers the density of the mineral particles that it is bound to by increasing the volume to mass ratio (Sollins *et al*, 2009). Crow *et al* (2007) gives a detailed account of the development of the density fractionation technique. In summary: density separation has progressed from its simplest two-pool form (light fraction (LF) and heavy fraction (HF)), through different separation solutions including bromoform, sodium iodide (NaI) and SPT (SOMETU, Berlin), and the addition of a sonication step to disrupt aggregates and isolate an additional pool (the intra-aggregate LF) after removal of the ‘free’ LF.

The currently preferred medium for density separation is SPT as it is non-toxic, contains no C compounds, does not become viscous at high densities, and can be relatively easily recycled and re-used (Six, *et al*, 1999b; Morgun and Makarov, 2011). However, the Na^+ in SPT disassociates in solution (Six, *et al*, 1999b; Morgun and Makarov, 2011), contaminating

the isolated fractions. For this reason the isolated fractions must be thoroughly rinsed with a CaCl solution after exposure to SPT to remove the Na⁺ ions. These dissociated Na⁺ ions can also cause the redistribution of highly negatively charged colloids (as discussed above, Kaiser and Guggenberger, 2007), causing them to be isolated in lighter fractions.

The amount of LF recovered during density separation is highly sensitive to the density of the liquid and so the density separation solution must be exactly adjusted to the desired density, with the aim of extracting the minimum amount of mineral material possible and limiting the allocation of SOM to incorrect pools and misinterpretation of SOM pool characteristics (Cerli *et al*, 2012). Any moisture loss or input must be avoided and temperature should be regulated to prevent fluctuations in density (Christensen, 2001). Cerli *et al* (2012) recommended an optimum density of 1.6 g cm⁻³ density separation liquids as they found that there was incomplete separation of free OM at lower densities and that more mineral material was floated at greater densities.

There are several studies, including John *et al* (2005), Sollins *et al* (2006), Sollins *et al* (2009) and Basile-Dolsch *et al* (2009), which use sequential density fractionations – repeated fractionations with increasing SPT density after removal of lighter SOM by aspiration – to yield fractions (six in the cited cases) of SOM attached to minerals. However, the SPT densities used were often of different ranges, and some sonicated samples after removal of the lightest fraction to isolate the intra-aggregate LF (Sohi *et al*, 2001; Wagai *et al*, 2008; Basile-Dolsch *et al*, 2009). Including the sonication step is favourable as the intra-aggregate LF is expected to be more decomposed than the free LF, have different chemical characteristics (Sohi *et al*, 2001) and, as it has a greater level of protection, a longer mean residence time than the free LF but shorter than the mineral bound fraction (Burns, 2011).

A drawback to soil fractionation by density separation is that a significant portion of the OC can be dissolved in the density separation medium. Although this C can be quantified by subtraction, it cannot be characterised. Schulze *et al* (2009) found that 2–6% of OC could be dissolved in SPT. It is also possible that not the entire light fraction is removed on density separation, leading to an underestimate in the light fraction and an overestimate in the heavy fraction C stocks.

For the purposes of studying the impact of tillage on soil aggregation, Six *et al* (1998) developed a method combining particle-size and density separation, whereby soil was first wet sieved to four different size categories, then all but the smallest size (< 53 µm) were density separated in SPT at 1.85 g cm⁻³. The resulting three heavy fractions were then dispersed by shaking in hexametaphosphate before further size fractionation. This procedure resulted in 12 separate fractions.

3.1.4 Chemical fractionation

The hydrolysis of SOM using acids and bases in soil fractionation operates on the principle that labile material will be readily solubilised and the recalcitrant material will remain in the residue; treatments are normally applied to the mineral bound (heavy) fractions (Trumbore and Zheng, 1996). Comparing chemical analyses of the residues and untreated fractions provides information on the reactive portion of the heavy SOM fractions. Helfrich *et al* (2007) compared five different chemical fractionation methods. They found that none of the tested methods (oxidation with hydrogen peroxide, sodium hypochlorite (NaOCl) or disodium peroxodisulphate, step wise hydrolysis, or demineralisation (treatment of NaOCl resistant fraction with hydrochloric acid)) were effective at completely removing all reactive OC but that the hydrogen peroxide and disodium peroxodisulphate were the most effective. However, Mikutta *et al* (2006) used radiocarbon dating to show that NaOCl removes a chemically reactive fraction of C that has a relatively short turnover time and leaves behind OM which has been stabilised by association with mineral surfaces (Fe and Al (hydr)oxides).

Trumbore and Zheng (1996) found that acid (0.5 M HCl) followed by base (0.1 M sodium pyrophosphate) hydrolysis removed more C than if followed by strong acid under heat (6 M HCl at 95 °C). They recommended a fractionation procedure which combines both density and chemical fractionation.

3.2 Fractionation methods

There are a wide variety of different procedures in the literature using either one or a combination of the above techniques and also variations in the parameters of the procedures (i.e. aggregate sizes, density of 'light' material and oxidising chemicals used in chemical fractionation).

The plethora of different fractionation procedures presents a problem when trying to draw conclusions across different studies (Cerli *et al*, 2012). To give some sense of the variety and connectedness of the methods I will outline an example. The sequential density separation method of Basile-Doelsch *et al* (2007) isolated 11 separate fractions with the purpose of studying the mineral bound fractions (of which there were eight, all within different density ranges). Basile-Doelsch *et al* (2007) has subsequently been cited 55 times (by 28/06/2016 according to Web of Science) and the method used un-adapted by Basile-Doelsch *et al*, (2009), adapted by Rumpel *et al* (2012) (isolating two fractions), Bonnard *et al* (2012) (isolating seven fractions), de Junet *et al* (2013) and El-Mufleh *et al* (2014) (isolating five fractions). In all of these adaptations, except

Rumpel *et al* (2012) Isabelle Basile-Doelsch is a named author. The method of Basile-Doelsch *et al* (2007) was itself adapted from the method of Baisden *et al* (2002) which in turn was adapted from the classic method of Golchin *et al* (1994). There are also a variety of other similar sequential density separation methods some of which are cited and discussed in section 3.1.3.

The following three methods were selected for comparison. The Sohi *et al* (2001) method was chosen as it is a commonly used, relatively simple method which is very similar to the method of Golchin *et al* (1994). The Zimmermann *et al* (2007) method was chosen as the authors calculated splitting factors to allow the masses of the isolated fractions to be used as input to initiate the RothC soil C dynamics model. The Ghani *et al* (2003) method was chosen as the fractions isolated are deemed to be highly sensitive to land-use change.

3.2.1 Sohi *et al* (2001)

The Sohi method is a two-step density separation method with dispersal by sonication being carried out after the free LF has been removed (Figure 3.1). Soil fractionated using this procedure were sieved to < 4 mm and air dried. Before fractionation the moisture was adjusted to 15% gravimetric moisture content following the capillary method of Haney and Haney (2010): around 20 g of air dried soil was placed into pre-weighed 30 ml plastic cups which had four 1.5 mm holes punched into the bottom of them and a Whatman No.1 Qualitative filter paper (cut to size) placed in the bottom to prevent soil loss. The plastic cups were then placed into glass jars containing 30 ml of deionised water, the jars were sealed to prevent evaporation and left until the surface of the soil appeared moist. The plastic cups were then removed from the jars, weighed and placed in a 40 °C oven. The mass of each cup was monitored until the soil had dried to 15% moisture content, after which they were removed from the oven and the soil stored in sealed plastic bags in a 3 °C fridge. Fractionation was carried out within 1 week of moisture adjustment.

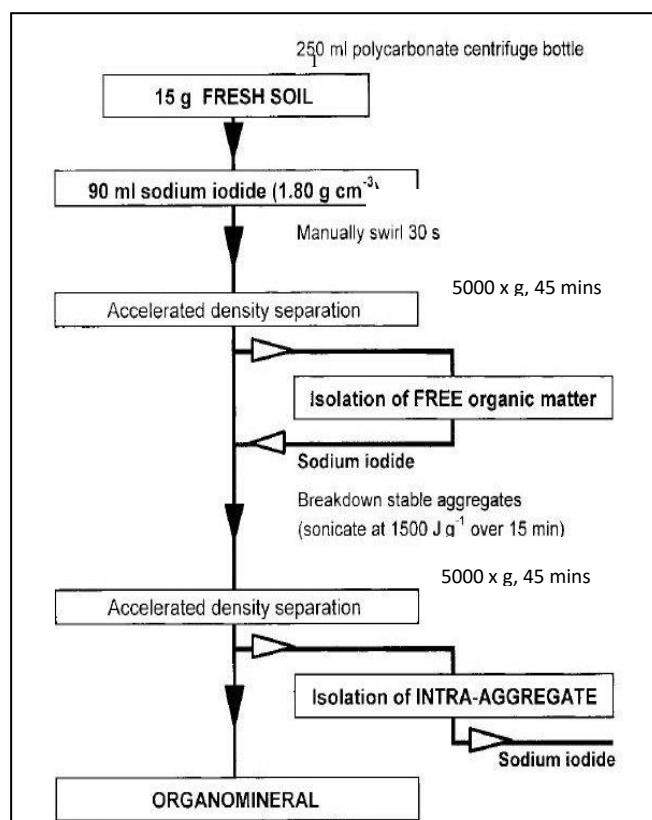


Figure 3.1: Flow diagram of the Sohi *et al* (2001) fractionation method.

Moisture adjusted soil (15 g) was placed in to a pre-weighed 250 ml polypropylene centrifuge bottle and then suspended in 90 ml NaI solution at a density of 1.81 g ml^{-1} by gently swirling the bottles for 30 seconds, both clockwise and anti-clockwise. The moisture in the soil lowered the density of the NaI to 1.80 g ml^{-1} . The bottles were then centrifuged for 45 minutes at $\sim 5000 \times g$ to accelerate sedimentation of heavy particles. After centrifugation, the supernatant was siphoned off using a vacuum pump, vacuum flask, a pipette and 6 mm tubing. The floating material was removed from the NaI by passing the solution through a vacuum filtration unit (Millipore, Hertfordshire, UK) with a nylon Whatman filter paper ($5 \mu\text{m}$, 90 mm diameter), the material left on the filter paper was the free LF.

The density of the siphoned off NaI was checked to ensure that it was still 1.80 g ml^{-1} (and carefully readjusted by adding small amounts of NaI if the density had decreased) before re-suspending the sediment in the centrifuge bottle. The sediment was then dispersed by sonication using a calibrated Soniprep 150 plus ultrasonic disintegrator (MSE (UK) Ltd, London) with an output energy of 1500 J g^{-1} . This output energy breaks apart labile aggregates whilst leaving sand sized particles intact (Monrozier *et al*, 1991). After centrifugation, the intra-aggregate LF is then obtained in the same way as the free LF. The residue remaining in the centrifuge bottle is the organo-mineral fraction.

The free LF and intra-aggregate LF were rinsed thoroughly in the filtration unit, firstly with 0.1 M CaCl solution (to release Na^+ ions) and then with at least 500 ml of deionised water,

before flushing the material from the filter paper in to a pre-weighed petri dish using deionised water and a wash bottle. The organo-mineral fraction was rinsed by adding ~150 ml of 0.1 M CaCl to the fraction in the centrifuge bottle, swirling, allowing the fraction to settle and siphoning off the CaCl. This process was repeated 3 subsequent times with deionised water. Fractions which were used in incubation experiments (Chapter 6) were rinsed continuously until the deionised water ran completely clear. All of the fractions were then weighed after being dried in a 40 °C oven.

Unfortunately, any readily soluble C is lost in the NaI solution and the deionised water used in rinsing the fractions. Although this uncaptured C pool cannot be characterised, its mass and C content can be estimated by subtracting the sum of the free LF, intra-aggregate LF and organo-mineral fraction masses and C contents from the corresponding whole soil values.

3.2.2 Zimmermann *et al* (2007)

The Zimmermann method is a combined physical and chemical fractionation method. Sonication is carried out on the whole soil at the beginning of the procedure (see Figure 3.2). This procedure holds merit in that it is a closed system and so all of the soil is accounted for at the end of the fractionation process. Zimmermann *et al* (2007) found that the SOC fractions isolated using this method correlated well to pools in the RothC model (Coleman and Jenkinson, 2014).

All soil fractionated using this method was prepared by sieving to < 4 mm and drying in a 40 °C oven. Soils were stored in sealed plastic bags until required for fractionation.

Deionised water (161 ml) was added to 30 g of soil in a 250 ml glass beaker. The soil was then sonicated, as described for the Sohi method, and wet sieved through a 64 µm sieve using a pressurised sprayer. All of the water and material (dark water) passing through the sieve was captured and transferred into a 2 l bottle. The volume of the dark water was recorded and the material left in the sieve was transferred into a pre-weighed mesh bag (pore size 60 µm), which was then placed in a 40 °C oven to dry.

Once dry, the mesh bag was re-weighed and the material was transferred into a centrifuge bottle along with 90 ml SPT solution at a density of 1.80 g cm⁻³. The POM fraction was then removed by centrifuging, siphoning and filtration as described for the Sohi method but using a 0.45 µm nylon filter (Whatman) in the filtration unit. The sediment remaining in the centrifuge bottle is the sand and stable aggregate (S+A) fraction; the S+A fraction was rinsed in the same way as the organo-mineral fraction in the Sohi method and then flushed into a pre-weighed petri dish.

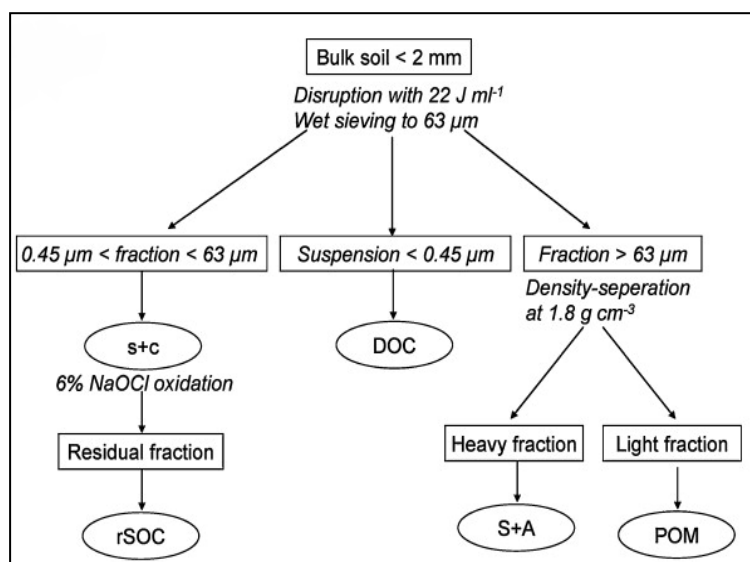


Figure 3.2: Flow diagram of the Zimmermann *et al* (2007) fractionation method. s+c – silt and clay, rSOC – residual soil organic C, DOC – dissolved OC, S+A – sand and aggregates, POM – particulate OM.

The dark water was decanted into a pre-weighed 250 ml centrifuge bottle and centrifuged for 15 minutes at $\sim 3500 \times g$. The supernatant was poured into a separate 2 l bottle, and the centrifuge step was repeated until all of the dark water had been centrifuged, and all of the soil material was rinsed out of the 2 l bottle. The centrifuge bottle is then dried at 40°C and re-weighed, giving the mass of the silt and clay (s+c) fraction.

However, there was a component of the s+c fraction remaining in the water after centrifugation. To quantify this, a subsample of the water was filtered through a pre-weighed Whatman nylon filter ($0.45 \mu\text{m}$ pore size), the filter was dried (at 40°C) and re-weighed, the mass of s+c per ml of water was then calculated and added to the mass of s+c fraction in the centrifuge bottle.

The filtered subsample of the centrifuged water was retained to determine dissolved organic C (DOC) content using a Rosemount-Dohrmann DC80 total C analyser (Rosemount Analytical, Santa Clara, CA).

The resistant soil organic C (rSOC) fraction is quantified by chemical oxidation of the s+c fraction. A 6% sodium hypochlorite (NaOCl) solution is allowed to react with a 1 g subsample of the s+c fraction for 18 hours in a pre-weighed centrifuge tube. The samples were then rinsed using deionised water and dried in a 40°C oven. The oxidation reaction was repeated twice, after which the centrifuge tube was dried and re-weighed. The proportion of the s+c fraction which is resistant can then be calculated from the proportion of the subsample which was not oxidised.

3.2.3 Ghani *et al* (2003)

The Ghani *et al* (2003) method has been used widely in the literature to assess the quality of forest and agricultural soils (e.g. Landgraf *et al*, 2006; Balaria *et al*, 2008; Bu *et al*, 2010; Spohn and Giani, 2011 and Uchida *et al* 2012). This method is different from the others as it separates only the hot water extractable fraction, which has been correlated with several soil and soil microbial properties (Ghani *et al*, 2003). Of particular interest here is the use of the hot-water extractable fraction as an indicator of the labile SOM content of the soil (Landgraf *et al*, 2006), and so the volume of soil C at risk of loss through microbial decomposition.

Hot-water extractable C (HWE) is considered to be sensitive to land-use change and so quantifying this soluble pool could be used as a cheaper and more rapid alternative to SOM fractionation techniques for monitoring the status of soil C stocks (Ghani *et al*, 2003). In this thesis the mean residence times, chemical characteristics and C concentrations of the water soluble C (WSC) and HWE C fractions will be compared to Sohi and Zimmermann fractions isolated from the same soil (Chapter 4).

Soils were prepared by sieving to < 4 mm and oven dried at 40 °C. Soil samples (3 g) were weighed into 50 ml centrifuge tubes and 30 ml deionised water was added (Figure 3.3). The centrifuge tubes were then placed on an orbital shaker (200 rpm for 30 minutes) in a temperature controlled room at 20 °C. The samples were then centrifuged at 4000 x g for 15 minutes and filtered through a 0.45 µm polyethersulfone filter membrane (Pall, 47 mm diameter) and the supernatant was decanted into a scintillation vial. This is the water soluble C (WSC). The centrifuge tubes were placed in a 40 °C oven until the sediment was dry.

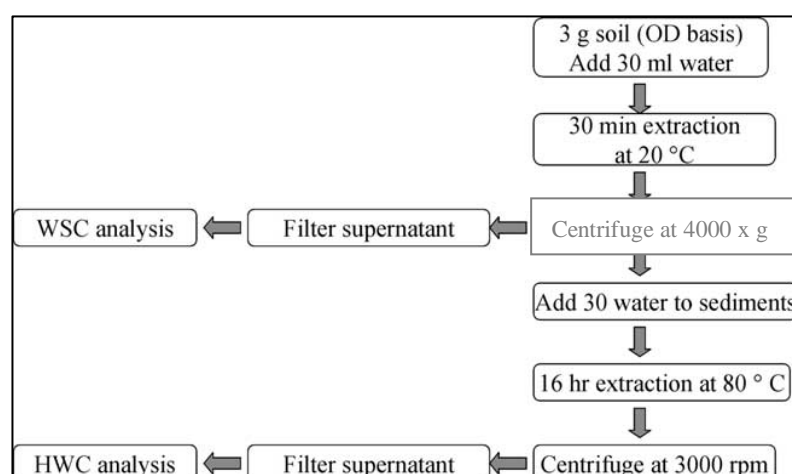


Figure 3.3: Flow diagram of the Ghani *et al* (2003) fractionation method. OD – oven dried, WSC – water soluble C, HWC – hot-water extractable C.

Once dry, a further 30 ml deionised water was added to the centrifuge tubes, which were then placed in an incubator at 80 °C for 16 hours. The centrifuge tubes were then placed on an orbital shaker (200 rpm for 30 minutes) and the hot water extractable C (HWE C) fraction was extracted in the same way as the WSC fraction.

The WSC and HWE C fractions were analysed for C content using a Rosemount-Dohrmann DC80 total C analyser (Rosemount Analytical, Santa Clara, CA).. The aromaticity of the WSC, HWE C and DOC fractions was estimated by determining ultra violet (UV) light absorbance values at 254, 465 and 665 nm using a Hewlett-Packard 8453 UV-vis spectrophotometer with 10 mm quartz cuvettes and using deionised water as a blank. Specific UV absorbance at 254 nm ($SUVA_{254}$) values and E_4/E_6 ratios were calculated. $SUVA_{254}$, expressed in units of $L\ mg\ C^{-1}\ m^{-1}$, is the average absorbance of all molecules in a liquid, calculated by multiplying the absorbance at 254 nm by 100 and normalising it for DOC concentration. $SUVA_{254}$ has been shown to be well correlated with DOC aromaticity (Weishaar *et al*, 2003). E_4/E_6 is the ratio of absorbance at 465 and 665 nm, which has been shown to have an inverse relationship with the molecular weight and condensation (i.e. aromaticity) of soluble humic substances such as low molecular weight fulvic and humic acids (Stevenson, 1982).

4 Soil Fractionation: A Comparison of methods using radiocarbon dating and spectroscopic analyses.

4.1 Introduction

Soil carbon (C) dynamics models are useful tools for predicting the impacts of management and climate change on future soil C stocks, and therefore C stability. Process based models such as RothC (Coleman and Jenkinson, 2014) segregate soil organic matter (SOM) into operationally defined conceptual pools with different decomposition rates based on first-order kinetics. For example, the RothC model partitions SOM into five pools: organic inputs are initially split into decomposable (DPM) and resistant (RPM) material. The DPM pool has a higher decomposition rate than the RPM pool. For each year modelled, a portion of each pool is lost as CO₂ and the remainder is split into either the microbial biomass (BIO) or the humified OM (HUM) pools. The BIO and HUM pools undergo further decomposition (at different rates), releasing a portion of CO₂ and separating into 'older' BIO and HUM pools. This cycle continues for a defined number of years. The fifth pool is an un-decomposable inert C pool (IOM) (Zimmermann *et al*, 2007).

These conceptual pools may be quantified in specific soils through the use of soil fractionation techniques. The question of whether we should 'measure the modellable or model the measurable' is central to development of models which can accurately predict SOC turnover. Elliott and Paustian (1996) suggested that soil C turnover models should be validated by measurable fractions. Sohi *et al* (2001) point out that it is impossible to isolate SOM with a homogenous turnover rate, this is because OM is a continuum of organic substances at various stages of decomposition rather than discrete homogenous pools (Lehmann and Kleber, 2015). Zimmermann *et al* (2007) developed a fractionation method which isolated fractions that correspond to pools in the RothC model.

Different soil fractionation procedures can yield different numbers of SOM pools (e.g. three for Sohi *et al* (2001), five for Zimmermann *et al* (2007), six for Six *et al* (2001), see Chapter 2 for a review of fractionation methods) of varying stability (Lefèvre *et al*, 2014), with mean residence times (MRTs) ranging from decades to thousands of years. The fractions isolated vary in size, ecological role and composition; similar fractions isolated by different methods may not fit the same conceptual definitions, which are dependent on the methods of separation used (Crow *et al*, 2007). Therefore care must be taken to ensure that the ecological properties of the isolated fractions are consistent with the conceptual definition and functionally defined conceptual SOM pools in models do not necessarily fit the same operationally defined pools isolated by fractionation. The wide use of different techniques in the literature presents a challenge when making comparisons across studies of

soil fractions: do similar fractions isolated by different methods fit the same conceptual definition?

The impacts of land management change on soil C stocks may take time to become apparent. Small changes in the short term may be masked by the background heterogeneity of the soil (Bolinder *et al*, 1999) however the ‘active’ labile C fractions are likely to show measurable changes in the short term (Conant *et al*, 2004; Franzluebbers and Stuedemann, 2002; Poeplau and Don, 2013; Plaza-Bonilla *et al*, 2014). Water soluble C (WSC) and hot-water extractable C (HWE) are components of the labile pool and considered to be sensitive to land-use change (Ghani *et al*, 2003). Quantifying these soluble pools could be used as a cheaper and more rapid alternative to SOM fractionation techniques for monitoring the status of soil C stocks (Ghani *et al*, 2003).

Radiocarbon (^{14}C) dating can be used to determine the rate at which C is cycling through SOM and is an important means by which SOM dynamics models can be tested and calibrated (Trumbore, 2009). Due to the relatively fast exchange of CO_2 between living plants and the atmosphere, the ^{14}C content of dead OM entering soil is comparable to that of the atmosphere (Marschner *et al*, 2008). The $^{14}\text{C}/^{12}\text{C}$ ratio in the OM decreases over time as the ^{14}C decays, comparing this to background atmospheric levels gives an estimate of the mean time since the C in the material was fixed from the atmosphere, known as a conventional ^{14}C age. Thermonuclear weapons testing in the 1950s and 60s caused the atmospheric ^{14}C concentration to almost double, reaching a high of 185 percent modern C (pMC), which has steadily decreased to the current level of 105.4 pMC (see Figure 4.1). This spike in atmospheric ^{14}C contents has allowed the proportion of the SOC that turns over on timescales of years to decades to be estimated. Models can then be applied to measured ^{14}C contents of whole soils and SOM fractions to estimate MRTs of SOC. MRTs are indicative of the stability of SOC as fractions with longer MRTs can be deemed to have greater stability than those with shorter MRTs.

Despite several literature reviews of soil fractionation methods (von Lützow *et al*, 2007; Marschner *et al*, 2008 and references in Chapter 3) a direct experimental comparison of commonly used fractionation methods employing different characterisation techniques (C and N analysis, ^{14}C dating and FT-IR analysis) has not previously been undertaken. This experiment set out to answer these specific questions:

1. For modellable SOM fractions isolated from the 0–10 cm layer of a managed grassland soil, what are the measured ^{14}C ages and MRTs and what do they mean in terms of C stability?

2. How do the ^{14}C ages and MRTs compare between fractions isolated using different fractionation techniques?
3. Do ‘modellable’ labile SOM/DOC fractions isolated from the 0–10 cm soil layer have similar MRTs to the labile SOC fractions (in the form of DOC) isolated using the simpler and cheaper hot-water extraction technique?

4.2 Methods

4.2.1 Soil and soil preparation

The soil used was a moderately well drained sandy loam grassland from the Crichton Royal Farm (Cottage Bottom field) in south-west Scotland (Latitude: N55:02:31 (55.041966), Longitude: W3:35:18 (-3.588243)). The field was permanent cut grassland which had not received lime for over 20 years and had no long-term history of organic manure application (Bell *et al*, 2015b). Soils belonged to the Holywood association and Crichton Midpark soil series (classified as Eutric Cambisols according to the FAO world reference base for soils (FAO, 1990)) which are non-calcareous and derived from red sandstone parent material.

Soil was collected from the Cottage Bottom field using an auger from 0–10 cm in the spring of 2012, sieved to < 4 mm, air dried and stored in sealed plastic bags. The soil had a pH of 6 and an OC content of ~3%.

4.2.2 Soil Fractionation

Soil samples were fractionated using three published methods which are used frequently in soil C cycling studies: the Zimmerman *et al* (2007) and Sohi *et al* (2001) density separation methods and the more simple hot-water extraction method (Ghani *et al*, 2003) as described in detail in Section 3.2. Isolated SOM fractions from replicate fractionation procedures were bulked after being dried at 40 °C then ball milled and placed in glass vials to be submitted for FT-IR or ^{14}C analysis. Liquid fractions were stored in glass bottles which had been rinsed with de-ionised water from the same source as was used in the fractionation procedure and air dried to ensure that there was no contamination of the samples.

4.2.3 Characterisation of whole soil and isolated fractions

Duplicate sub-samples of the fractionated whole soil and isolated SOM fractions were prepared for ^{14}C analysis at the NERC radiocarbon facility (East Kilbride, UK) by converting them into graphite targets through a process of combustion and cryogenic purification of the C, and then reduction using cobalt and iron. For liquid samples, prior to ^{14}C analysis, measured sample volumes were rotary evaporated to a few millilitres and then quantitatively transferred to pre-weighed, acid washed beakers. The samples, covered by glass fibre filter

papers, were then freeze dried and fumigated with HCl to hydrolyse any carbonates. ^{14}C analysis was carried out by accelerator mass spectrometry (AMS) at the Scottish Universities Environment Research Council (SUERC) AMS Laboratory (East Kilbride, UK).

Ball milled whole soil and solid fraction sub-samples were combusted and analysed for total organic C (OC) and total nitrogen (N) using a Flash 2000 elemental analyser. Zimmerman DOC, water collected during rinsing of the density separated Zimmermann fractions and Ghani WSC and HVEC were acidified with two drops of orthophosphoric acid, sparged with N_2 for two minutes to remove any carbonates and then analysed for DOC using a Rosemount-Dohrmann DC80 total C analyser.

The chemical characteristics of whole soil, solid SOM fractions and freeze-dried HVEC were determined using a Bruker Vertex 70 FT-IR spectrometer using a Diamond Attenuated Total Reflectance accessory at the James Hutton Institute. Sample spectra were acquired by averaging 200 scans at 2 cm^{-1} resolution over the range $4000\text{--}370\text{ cm}^{-1}$. Spectra were baseline corrected (see Palacio *et al* (2014) for further information).

FT-IR analysis was not carried out for the Ghani WSC or the Zimmerman DOC fractions as insufficient quantities of solid samples were obtained on freeze drying of the liquids. Instead, the aromaticity of the WSC, HVEC and DOC fractions was estimated by determining ultra violet (UV) light absorbance values at 254, 465 and 665 nm using a Hewlett-Packard 8453 UV-vis spectrophotometer with 10 mm quartz cuvettes and using deionised water as a blank. Specific UV absorbance at 254 nm (SUVA_{254}) values and E_4/E_6 ratios were calculated (see section 3.2.3).

A whole soil sub-sample was screened for black C by thermal gravimetric analysis (TGA) (TGA/DSC 1; Mettler-Toledo, Leicester, UK). The sample was combusted in air (using nitrogen as the protective gas) by heating from 35 to $650\text{ }^\circ\text{C}$, increasing at a rate of $10\text{ }^\circ\text{C}$ per minute. The mass of the sample was recorded every 0.025 minutes.

Whole soil and POM sub-samples were also screened for black C by hydropyrolysis (HyPy) at SUERC following the procedure in Meredith *et al* (2012). HyPy was used to isolate the thermally stable, black C fraction of the samples. HyPy uses high hydrogen gas pressures and a sulphided molybdenum catalyst during pyrolysis in order to achieve very high conversions of labile OM, leaving behind any refractory, highly aromatic carbonaceous material as a residue. The high hydrogen pressures, slow heating rate and catalyst suppress the neo-formation of any black C during the procedure, enabling highly accurate and reproducible black C determinations in a wide range of environmental matrices, including soils. Prior to HyPy, 100 mg aliquots of the samples were loaded with the catalyst by addition of an aqueous/methanol solution (80:20 v/v) of ammonium dioxidythiomolybdate

[(NH₄)₂MoO₂S₂]. The catalyst addition was 10% by weight. Catalyst loaded samples were weighed into shortened borosilicate pipette ends and placed within the HyPy reactor. The samples were pyrolysed with resistive heating from 50 to 250 °C at 300 °C min⁻¹, and then from 250 °C to 550 °C at 8 °C min⁻¹. The final temperature was held for 2 minutes under a hydrogen pressure of 150 bar and a hydrogen sweep gas flow of 5 L min⁻¹. The OC content of the samples before and after HyPy was used in order to calculate the black C content of the samples. OC was measured using a Costech Elemental Analyser (EA) coupled to a vacuum line. After combustion of the samples in the EA, CO₂ gas was collected cryogenically for measurement. The black C content of the samples was calculated as follows:

$$\text{Black C}_{\text{HyPy}} (\%) = \frac{\text{Residual OC (mg C in residue)}}{\text{Initial OC (mg C in sample)}} \times 100$$

4.2.4 Modelling mean residence times

Conventional ¹⁴C ages can be assigned according to ¹⁴C enrichment, but this requires a number of assumptions to be made and has a relatively high uncertainty associated with it (1 standard deviation) (Stuiver and Polach, 1977). However, conventional ¹⁴C ages cannot be directly interpreted as MRTs and so a modelling approach is employed to calculate these.

The model used to determine MRTs (following the method of Buckingham, 2008) is a simple one pool model which assumes that the C stocks are in a steady state i.e. C inputs to the system in the form of litter and root exudates is equivalent to C loss through decomposition. As the soil used in this experiment had been under grassland management since ~1843, and had no long-term history of organic inputs it was assumed to be in a steady state.

In order to estimate the C inputs to and MRTs of the whole soil and isolated SOM fractions, the model used methods similar to Harkness *et al* (1986) and Mills *et al* (2014) and is based on the following principles:

1. The measured ¹⁴C content (pMC) of each SOM fraction and whole soil sample is used to compare and constrain the calculated ¹⁴C with the measured value.
2. The ratio of C input added to the whole soil or C pool, as an approximation of C turnover.
3. The SOC stock, expressed in units of g m⁻² is used to estimate the C added to the top soil or to each fraction pool.
4. Microsoft Excel's Solver package is used to minimise the difference between calculated and measured soil ¹⁴C by changing the annual input to the soil C pool. In

addition Solver is used to constrain the model when estimating the quantity of added C to the soil. A realistic estimate is sufficient to prevent the model producing impractical outputs resulting from the opportunity of two outcomes, associated with the rise and fall of the bomb- ^{14}C peak (Figure 4.1).

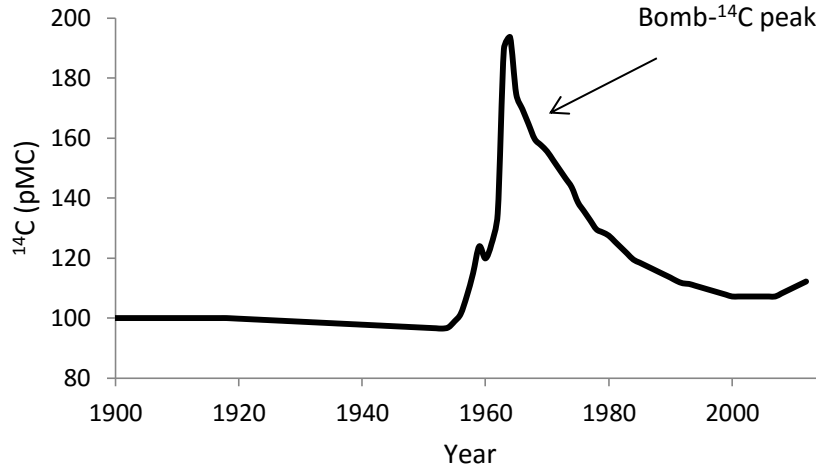


Figure 4.1: Atmospheric ^{14}C concentrations used in the model, in % modern C (pMC) from 1900 to 2012. Concentrations before 1900 were assumed to 100 pMC. The large bomb- ^{14}C peak is due to nuclear weapons testing around the 1950s and 1960s.

The model works on an annual time-step beginning in the year 1001, when the atmospheric ^{14}C was assumed to be 100% modern and the whole soil ^{14}C was approximately 99.5% modern. These values were chosen to initiate the model as they ensured that the model ‘spins-up’ to a steady state within a reasonable time frame. The model was used previously on the basis of ^{14}C entering the whole soil, whereas in this study we have applied the model to fractionated SOM pools. Transfers of carbon are calculated as follows:

$$\Delta^{14}\text{C} = \Delta\text{C} \times A^{14}\text{C}_i$$

where $\Delta^{14}\text{C}$ is the change in SOM fraction ^{14}C from year i to year $i+1$, ΔC is the estimated C added to the fraction and $A^{14}\text{C}_i$ is the atmospheric ^{14}C content in year i . However, as the soil is in a steady state, the ^{14}C leaving the whole soil or SOM fractions must also be calculated:

$$\Delta^{14}\text{C} = \Delta\text{C} \times f^{14}\text{C}_i$$

Where $f^{14}\text{C}_i$ is the SOM fraction ^{14}C content in year i . The ^{14}C content for the whole soil or fraction in year $i+1$ is then calculated as:

$$\text{Net } f^{14}\text{C}_{i+1} = \sum \left[\left(\text{SOC} \times f^{14}\text{C}_i \right) + \left(\Delta^{14}\text{C} \right) \right] / \text{SOC}$$

where SOC is the fraction C stock (g m^{-2}). However, a small proportion of the ^{14}C decays each year and so the net $f^{14}\text{C}_{i+1}$ is multiplied by a factor of 0.99988 to account for this. The model then runs through time-steps $i+2$, $i+3$ with appropriate adjustments being made to the atmospheric ^{14}C contents to account for the dilution of atmospheric ^{14}C caused by the magnitude of ^{14}C depleted CO_2 emissions from industrial burning of fossil fuels since the 1900s (Suess effect, Suess, 1955) and for the peak caused by bomb- ^{14}C after 1950. The model is run till the year ^{14}C measurements were taken providing an estimate of ^{14}C content.

Following this, the model is optimised using the Microsoft Excel Solver package through minimising the squared residual of the observed and calculated ^{14}C contents of the whole soil or fraction SO^{14}C for the particular year that ^{14}C was measured (2012 in this case). The turnover time of C for each whole soil or fraction was finally calculated through the following calculation:

$$\text{C turnover time (years)} = \frac{\text{C pool (g m}^{-2}\text{)}}{\text{Added C (g m}^{-2}\text{yr}^{-1}\text{)}}$$

4.3 Results

4.3.1 Fractionation efficiency and distribution of organic C and N between soil fractions

The Zimmermann *et al* and Sohi *et al* methods recovered $98.6 \pm 1.3\%$ and $95.2 \pm 0.4\%$ of the initial soil mass respectively (Figure 4.2). Recovery of OC and TN was $110.6 \pm 1.6\%$ and $90.2 \pm 0.8\%$, respectively, for the Zimmermann *et al* method and $94.0 \pm 0.7\%$ and $83.0 \pm 0.5\%$, respectively, for the Sohi *et al* method. An additional 1.1% of the initial whole soil OC was accounted for in the deionised water used to rinse SPT out of the Zimmermann density separated fractions, and it is assumed that the missing 4.8% of initial whole soil OC from the Sohi *et al* method was lost as dissolved OC in the NaI although this was not quantified. The Ghani *et al* method extracted 2.7% of whole soil OC, 2.4% of which was in the HWEF fraction.

The SOC stock for the Crichton whole soil was $24 \pm 1.5 \text{ tC ha}^{-1}$ (Table 4.1). As has been found in other studies (Zimmerman *et al*, 2007; O'Brien *et al*, 2013), the majority of OC was stored in the mineral bound fractions of the soil (Table 4.1 and Figure 4.2). For the Zimmermann *et al* method, the S+A fraction made up the bulk of the whole soil mass ($600 \pm 8 \text{ kg t}^{-1}$), and although the rSOC mass ($367 \pm 10 \text{ kg t}^{-1}$) was only around 60% that of the S+A, they had similar C stocks (10.2 ± 0.2 and $10.3 \pm 0.3 \text{ t C ha}^{-1}$, respectively).

Despite the POM and free LF fractions containing the greatest concentrations of C (~31 and ~13% of fraction mass, respectively) they make up only a small proportion of the whole soil mass (9.2 ± 1.3 and $14.5 \pm 1.4 \text{ kg t}^{-1}$) and so have only small C stocks relative to the whole soil (2.3 ± 0.3 and $1.9 \pm 0.2 \text{ t C ha}^{-1}$ respectively). The organo-mineral fraction made up the vast bulk of the Sohi fractions ($913 \pm 3 \text{ kg t}^{-1}$) and contributed the largest proportion of C. The C concentration of the fractions, however, decreased in the order free LF > intra-aggregate LF > organo-mineral fraction.

The C:N of the free LF and intra-aggregate LF fractions were significantly higher than the mineral bound and resistant fractions (one-way ANOVA, $p < 0.001$) (Table 4.1), in accordance with other studies (John *et al*, 2005, Golchin *et al*, 1994), indicating that these fractions are comprised of less decomposed material.

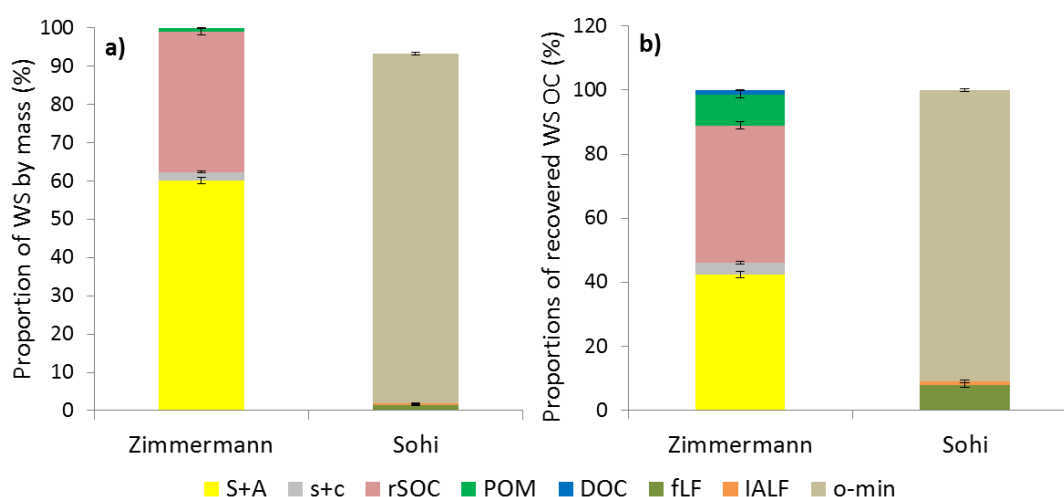


Figure 4.2: Relative proportions (%) of whole soil (WS) contributed a) by mass, and b) by organic C (OC) by fractions isolated using the Zimmermann *et al* (2007) and Sohi *et al* (2001) fraction methods. Note that by mass values do not add up to 100% as not all soil was recovered during the fractionation procedure; OC values do add up to 100% as they are calculated against the recovered mass. S+A – sand and aggregates, s+c – silt and clay, rSOC – residual soil OC, POM – particulate organic matter, DOC – dissolved OC, fLF – free light fraction, IALF – intra-aggregate LF, o-min – organo-mineral fraction.

Table 4.1: Mean relative masses (kg t^{-1}) and C stocks (t C ha^{-1}) and calculated C:N for whole soil and isolated SOM fractions from the Zimmermann *et al* (2007), Sohi *et al* (2001) and Ghani *et al* (2003) fractionation methods. Standard errors are given in parenthesis. Values with no letters in common after them are significantly different (one-way ANOVA followed by a Tukey post hoc test, $p < 0.05$). OM – organic matter, SOC – soil organic C, LF – light fraction.

	Fraction	Relative masses (kg t^{-1})	C stocks (t C ha^{-1})	N stock (t N ha^{-1})	C:N
Whole soil	-	1000	24.1 (1.5)	2.24	11.066 ^a
Zimmermann <i>et al</i> (2007)	Silt and clay	22.3 (2.6)	0.9 (0.1)	0.08 (0.01)	10.660 ^a
	Residual SOC	367 (10)	10.3 (0.3)	0.96 (0.03)	13.375 ^{ab}
	Sand and aggregates	600 (8)	10.2 (0.2)	0.95 (0.02)	12.538 ^{ab}
	Particulate OM	9.2 (1.3)	2.3 (0.3)	0.22 (0.03)	21.912 ^{bc}
	Dissolved OC	-	0.3	-	-
Sohi <i>et al</i> (2001)	Organo-mineral	913 (3)	21.9 (0.1)	2.17	11.990 ^{ab}
	Free LF	14.5 (1.4)	1.9 (0.2)	0.05 (0.01)	24.508 ^c
	Intra-aggregate LF	3.5 (1.3)	0.3 (0.1)	0.01	26.469 ^c
Ghani <i>et al</i> (2003)	Hot water extractable C	-	0.6	-	-
	Water soluble C	-	0.1	-	-

4.3.2 Isotopic signatures of the whole soil and isolated fractions

The mean ^{14}C contents of the duplicate whole soil and SOM fraction sub-samples (Table 4.2) are plotted in Figure 4.3, lower ^{14}C enrichment, expressed as percent modern C (pMC), equates to older C; values < 100 pMC suggest the material is likely to be composed mostly of pre-bomb C (pre-1950) whilst values > 100 pMC suggest the material contains post-bomb C, but may also contain some pre-bomb C. For the Zimmerman method, the fractions fell into two distinct age groups. The POM and DOC had the greatest ^{14}C ages whilst the s+c, rSOC and S+A fractions were relatively young. ^{14}C enrichment of the fractions decreased in the order s+c $>$ rSOC $>$ S+A $>$ POM $>$ DOC. These results were surprising, it had been expected that the DOC and POM fractions would be the youngest but both were significantly older (one-way ANOVA, $p < 0.001$) than the other Zimmerman fractions and the whole soil, but not significantly different from each other. Zimmerman *et al* (2007) found that the s+c fraction from a meadow soil had a ^{14}C enrichment value very close to that of the whole soil. However, they observed a greater reduction in ^{14}C content between the s+c and rSOC fractions than was achieved here (reduction of 11.76 compared to 4.37 pMC). O'Brien *et al* (2013) found that rSOC had a ^{14}C age of 2000–3000 years, 10 times greater than that found

here. These findings suggest that the NaOCl oxidation step did not fully remove labile OC in this study.

For the Sohi *et al* (2001) method, ^{14}C enrichment declined in the order organo-mineral > free LF > intra-aggregate LF, although it had been expected that the free LF and intra-aggregate LF would be younger than the organo-mineral fraction (Burns, 2011; Schrumpf and Kaiser, 2015). All fractions were significantly different from each other. The free LF and intra-aggregate LF were significantly different from the whole soil (one-way ANOVA, $p < 0.001$).

Labile fractions have generally been found to have ^{14}C contents which suggest they contain mostly ‘modern’ C (Leifeld *et al*, 2009). The unusually low ^{14}C values in the labile fractions (POM, DOC, free LF and intra-aggregate LF) coupled with high C:N are indicative of the presence of black C fragments in the soil (Glaser *et al*, 2000; Hamer *et al*, 2004; Brodowski *et al*, 2006). Brodowski *et al* (2006) found that a large proportion of black C was occluded within aggregates. In contrast the s+c and rSOC fractions were much younger than expected and were not significantly different from the whole soil or the S+A fraction.

In contrast to the ^{14}C contents, $\delta^{13}\text{C}$ values were as expected, with the labile fractions (POM, DOC, free LF and intra-aggregate LF) being more ^{13}C enriched than the mineral bound fractions and similar to the WSC and HWEF fractions. However, $\delta^{13}\text{C}$ didn’t vary much between fractions (-26 to -28.5 ‰). Zimmermann *et al* (2007b) found that rSOC for a meadow site was less ^{13}C enriched compared to the s+c fractions. In this study the two fractions had almost identical $\delta^{13}\text{C}$ contents, suggesting that the NaOCl oxidation was not successful in removing the labile component.

There was a significantly negative correlation between the C:N of isolated SOM fractions and ^{14}C contents ($R^2 = 0.72$, linear regression $p < 0.001$) and a significantly positive correlation between C:N and ^{13}C enrichment ($R^2 = 0.74$, linear regression $p < 0.001$). This contradiction may be due to a large proportion of labile ^{13}C enriched material and a small proportion of ^{14}C depleted black C in the labile Zimmermann and Sohi fractions.

The Ghani HWEF and WSC fractions had ^{14}C contents of 108.8 and 100.9 pMC, respectively, suggesting that they each contained predominantly ‘modern’ C with the WSC fraction containing a higher proportion of pre-bomb C than the HWEF fraction, consistent with results found for a forest soil (0–5 cm sampled in summer) by Nakanishi *et al* (2014); both fractions being more ^{14}C enriched than the whole soil (Table 4.2, Figure 4.3).

Table 4.2: Duplicate sub-sample ^{14}C enrichment expressed as % modern C (pMC), conventional ^{14}C age (years BP), $\delta^{13}\text{C}$ (‰) values and modelled mean residence times (MRTs) for whole soil and isolated SOM fractions from the Zimmermann *et al* (2007), Sohi *et al* (2001) and Ghani *et al* (2003) fractionation methods. Errors, shown in parenthesis, are associated with analytical confidence and represent 1 standard deviation. Values with different letters after them are significantly different (one-way ANOVA followed by a Tukey post hoc test, $p < 0.05$). SOC – soil organic C, OM – organic matter, LF – light fraction.

	Fraction	^{14}C enrichment (pMC) ($\pm 1\sigma$)	Conventional ^{14}C age (years BP) ($\pm 1\sigma$)	$\delta^{13}\text{C}$ (‰)	MRTs (years)	SUERC sample IDs
Whole soil	-	96.9 (0.44) ^c	255 (37)	-28.4 ^a	562.9	SUERC-51662
		95.5 (0.44)	370 (37)	-28.1	732.7	SUERC-51663
Zimmermann <i>et al</i>	Silt and clay	101.5 (0.46) ^c	Modern	-28.2 ^a	267.1	SUERC-51664
		100.5 (0.46)		-28	310.9	SUERC-51665
	Residual SOC	97.0 (0.42) ^c	248 (35)	-28.1 ^a	552.9	SUERC-51666
		96.3 (0.44)	305 (37)	-28.1	628.1	SUERC-51667
	Sand and aggregates	90.4 (0.39) ^{bc}	809 (35)	-28.5 ^a	3461 [*]	SUERC-51670
		85.8 (0.37)	1234 (35)	-27.9	-2915 [*]	SUERC-51671
	Particulate OM	60.7 (0.28) ^a	4016 (38)	-26.5 ^{bc}	-505.4 [*]	SUERC-54820
		58.4 (0.27)	4315 (37)	-26.7	-484.1 [*]	SUERC-54821
Sohi <i>et al</i>	Dissolved OC	54.1 (0.25) ^a	4937 (37)	-27.0 ^{ab}	-451.5 [*]	SUERC-54829
		40.4 (0.20)	7272 (39)	-27.4	-384.2 [*]	SUERC-54830
	Organo- mineral	97.7 (0.45) ^c	190 (37)	-28.0 ^a	489.2	SUERC-51672
		97.3 (0.45)	223 (37)	-28.3	524.3	SUERC-51673
	Free LF	78.6 (0.36) ^b	1932 (37)	-27.7 ^{ab}	-985.4 [*]	SUERC-54822
		78.0 (0.36)	1998 (37)	-27.1	-943.6 [*]	SUERC-54823
	Intra- aggregate LF	45.2 (0.23) ^a	6376 (24)	-26.0 ^c	-403.3 [*]	SUERC-54824
		47.6 (0.22)	5968 (38)	-26.1	-414.4 [*]	SUERC-54825
Ghani <i>et al</i>	Hot water extractable C	108.6 (0.5) ^c	Modern	-27.0 ^{bc}	100.2	SUERC-54833
		109.0 (0.5)		-26.8	95.1	SUERC-54834
	Water soluble C	101.7 (0.47) ^c	Modern	-26.4 ^{bc}	259.2	SUERC-54831
		100.1 (0.46)		-26.6	330.8	SUERC-54832

* These MRTs are unrealistically high or low as a consequence of the modelling procedure and presumably the presence of black C

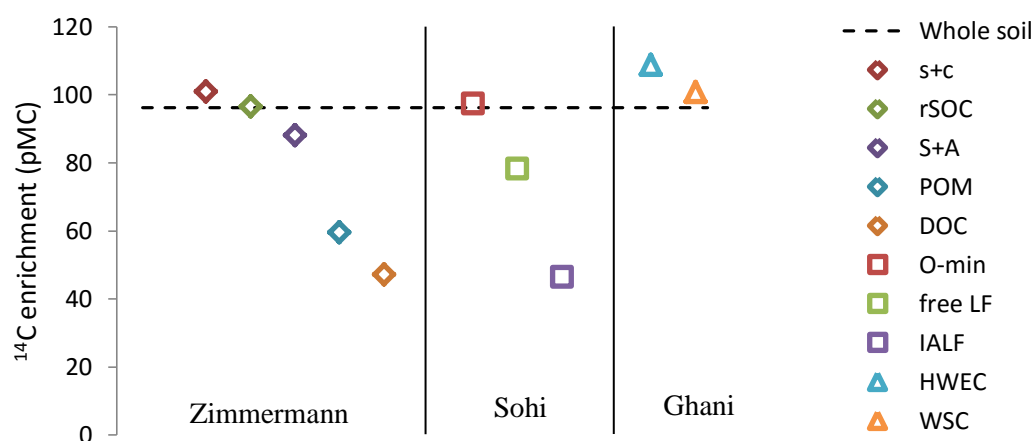


Figure 4.3: Mean ^{14}C enrichment values expressed as % modern C (pMC) for isolated SOM fractions from the Zimmermann *et al* (2007), Sohi *et al* (2001) and Ghani *et al* (2003) fractionation methods relative to the whole soil. s+c – silt and clay, rSOC – residual soil organic C, S+A – sand and aggregates, POM – particulate organic matter, DOC – dissolved organic C, O-min – organo-mineral fraction, LF – light fraction, IALF – intra-aggregate LF, HWEC – hot-water extractable C, WSC – water soluble C.

4.3.3 Mean residence times

For the whole soil, Zimmermann s+c and rSOC fractions and the Sohi organo-mineral fractions, MRTs were within expected ranges. Grassland and forest soils have MRTs, estimated using bomb ^{14}C , ranging from 36 to 1542 years (mean 535 ± 134 years) (Six and Jastrow, 2006). The average MRTs for the s+c and organo-mineral fractions were 289.0 ± 21.9 and 506.8 ± 17.6 years, respectively, which is within the range found by Wattel-Koekkoek *et al* (2003) for a variety of clay (kaolinite) associated C samples (average of 357 ± 121 years) and by Schrumpf and Kaiser (2015) who calculated a MRT of 115–625 years (0–5 and 10–20 cm, respectively) for the heavy fraction of a forest soil. In this study, the rSOC fraction had a ^{14}C content which was not significantly different from the s+c but had a slightly older MRT (590.5 ± 37.6 years) which is consistent with the loss of a labile component from the s+c fraction on hydrolysis (Wattel-Koekkoek *et al*, 2003).

The HWEC fraction had the lowest MRT (97.6 ± 2.6 years), consistent with the extraction of only labile and non-physically or chemically protected SOC.

Soil fractions which had unusually low ^{14}C contents (Zimmermann POM, S+A and DOC and Sohi free LF and intra-aggregate LF) had negative or very high (3461) MRTs (Table 4.2). von Lützow *et al* (2007) summarised typical MRTs for density fractions from different studies and found that light fraction MRTs ranged from 1 year (free LF) to 83 years (occluded POM). The negative and very large MRTs found here are a consequence of the modelling procedure (see Section 4.3.4) because the measured ^{14}C values were below recorded atmospheric concentrations (Figure 4.1) and the model assumes a small annual loss of C to reproduce this (see Appendix 2 for model outputs). The model was further

constrained to prevent negative C input values but this did not eliminate impractical model outputs. The initial soil ^{14}C content was also adjusted to be more similar to the measured ^{14}C , this produced high positive MRTs. Due to the nature of the bomb C peak, measured ^{14}C may refer to two possible dates that lie on either the rise or fall of the bomb- ^{14}C peak. (e.g. a ^{14}C content could relate to OM deposited to the soil around either 1957 or 1992, See Figure 4.1).

Inputs of bomb- ^{14}C enriched material to these fractions are likely to become diluted by the black C (due to the small pool sizes) and so are not evident in the ^{14}C signature, causing the model to select the lower of the two possible dates. It is apparent that the model used to estimate MRTs is not sophisticated enough to account for low ^{14}C contents or the possibility of black C within SOC pools.

4.3.4 Chemical characterisation of whole soil and SOM fractions

FT-IR analysis

FT-IR spectra for the whole soil, HWEC and all Zimmerman and Sohi fractions are shown in Figures 4.4, 4.5 and 4.6, with the four major absorption bands (hydroxyl, aliphatic, aliphatic carboxylate/aromatic and polysaccharide/silicate groups) indicated. The broad absorption band around 3400 cm^{-1} , attributed to H bonded OH groups of OM and/or on mineral (e.g. kaolinite) surfaces, was well pronounced in free LF (Figure 4.5) and POM (Figure 4.4). The sharpness of the 3400 cm^{-1} band in the intra-aggregate LF (Figure 4.5) fraction indicates dominance of the kaolinite surface OH doublet rather than other mineral or organic OH groups in this fraction. The fractions with higher OC concentrations (Zimmerman POM (Figure 4.4), Sohi free LF and intra-aggregate LF (Figure 4.5)) had more pronounced absorption for the aliphatic C-H stretching groups (2920 and 2850 cm^{-1}) and aliphatic carboxylates and/or aromatic group bands (around 1610 cm^{-1}) than the whole soil and the mineral bound fractions. The 1610 cm^{-1} band was particularly strong in the intra-aggregate LF fraction (Figure 4.5). Strong absorption bands around 1000 cm^{-1} indicated C-O stretching of polysaccharide or polysaccharide-like substances and/or Si-O stretches of silicates (if minerals were present) in the whole soil and all fractions. This absorption band was relatively weaker in the intra-aggregate LF fraction (Figure 4.5) compared to the other fractions and whole soil indicating greater humification in this fraction. Peak assignments were according to Palacio *et al*, 2014.

The greater aromatic, carboxyl, carboxylate (Sohi *et al*, 2001), alkyl and carbonyl C functional groups (John *et al*, 2005) and less intense polysaccharide bands (Sohi *et al*, 2001) in the intra-aggregate LF fractions than in the free LF (Figure 4.5) indicate greater humification in this fraction. This has also been observed in other studies for sandy loam (Sohi *et al*, 2001), silty-clay loam (Sohi *et al*, 2001; Poirer *et al*, 2005), and silty loam (John *et al*, 2005) agricultural soils.

The HWEC fraction was the only dissolved C fraction for which an FT-IR spectrum could be obtained, and the chemical characteristics were somewhat different to the solid samples (see Figure 4.6). The 3400 cm^{-1} band was particularly strong and masked the aliphatic groups at 2850 and 2920 cm^{-1} . The aliphatic carboxylate and/or aromatic group absorption bands at 1610 cm^{-1} were also relatively stronger in the HWEC fraction than the other fractions and whole soil.

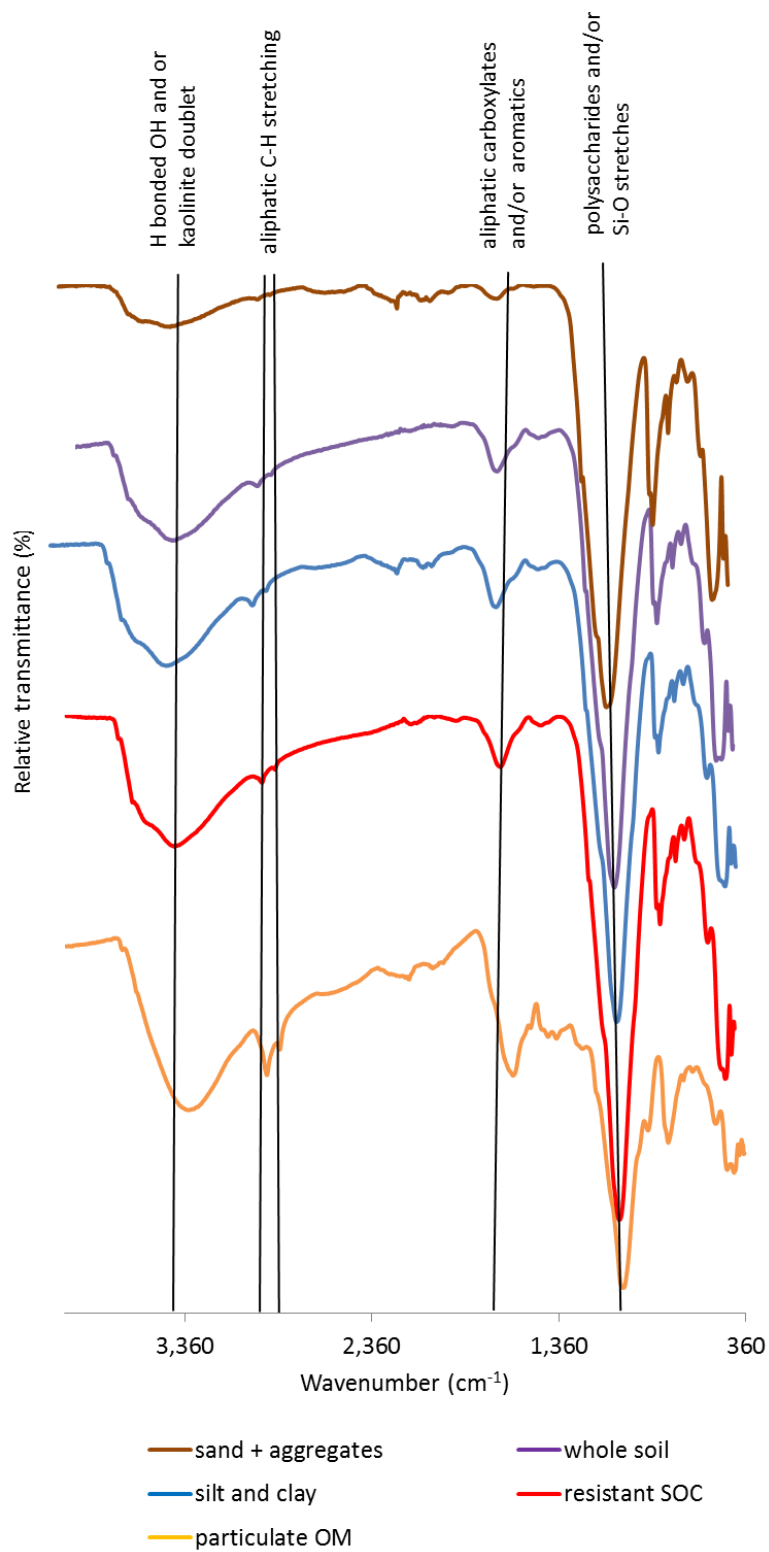


Figure 4.4: FT-IR spectra for soil organic matter fractions isolated using the Zimmermann *et al* (2007) fractionation method and whole soil. SOC – soil organic C, OM – organic matter.

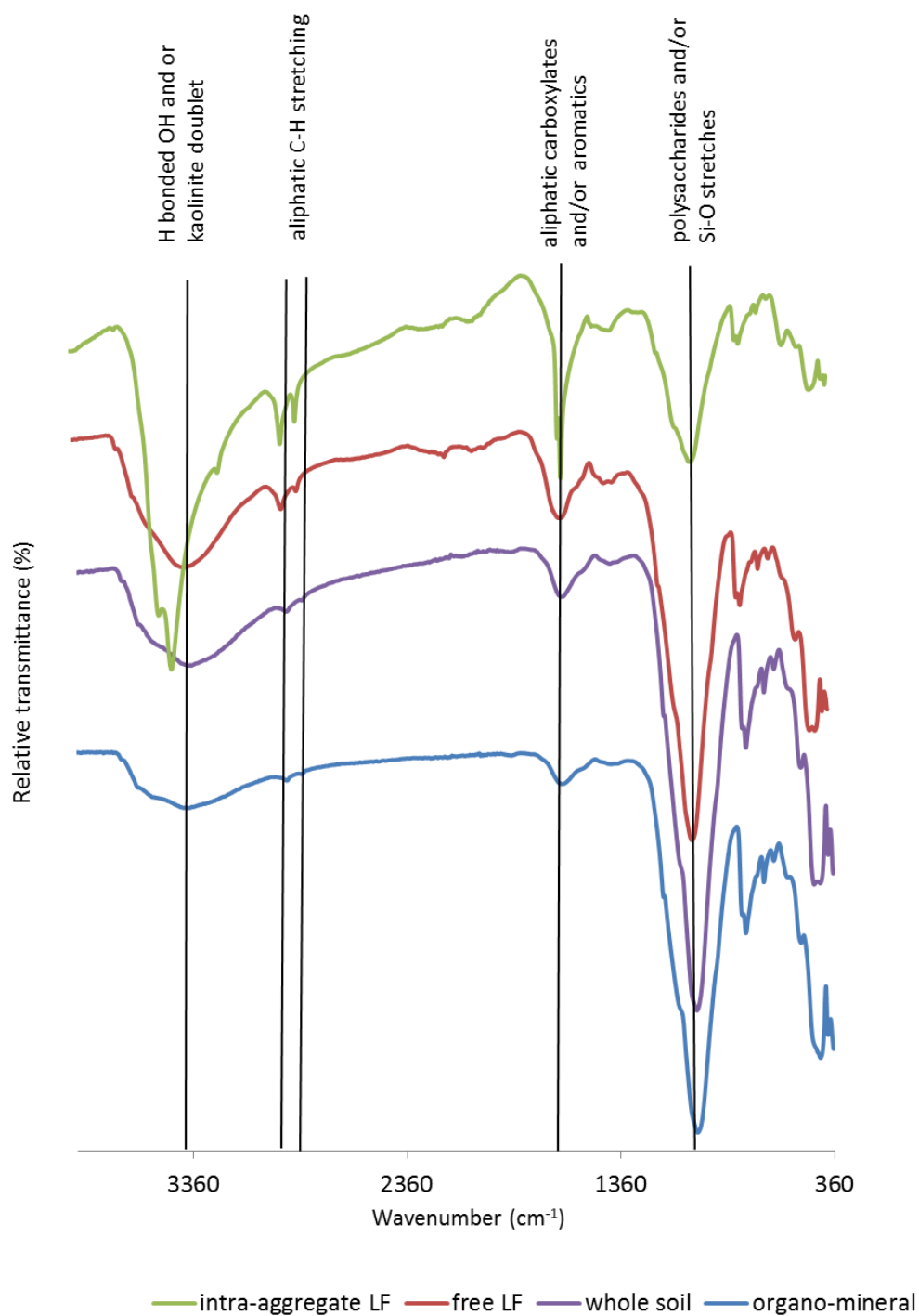


Figure 4.5: FT-IR spectra for soil organic matter fractions isolated using the Sohi *et al* (2001) fractionation method and whole soil. LF – light fraction.

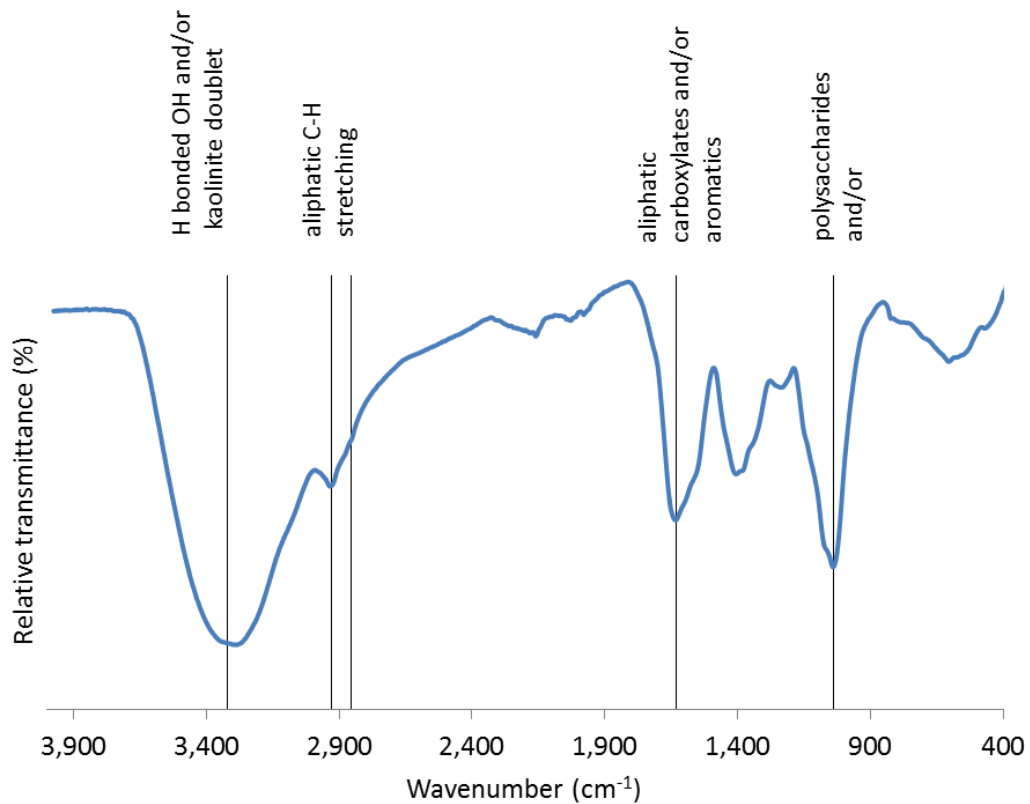


Figure 4.6: FT-IR spectra for hot-water extractable C fraction isolated using the Ghani *et al* (2003) method.

It had been expected that oxidation of the s+c fraction would result in the loss of carboxyl, aromatic and aliphatic functional groups (Zimmerman, 2007; Mikutta *et al*, 2005), however s+c and rSOC fraction absorption band strengths for these groups showed very little difference (Figure 4.7). Mikutta *et al* (2005) achieved a 49–81% oxidation of OC from the s+c using NaOCl compared to an average of 27.4% in this study. The absence of any strong oxidation effect may have been due to the concentration of the NaOCl being too low (5%), and so not effectively oxidising all chemically reactive SOM. The FT-IR spectra provide some evidence to support this, although the slight increase in C:N and decrease in ^{14}C enrichment of the rSOC fraction relative to the s+c would suggest that some oxidation did occur.

Note that organic functional group absorption bands in the whole soil and mineral bound fractions (S+A, s+c and organo-mineral) FT-IR spectra are likely to be masked by absorption bands for mineral components (e.g. Si-O groups at $\sim 1000\text{ cm}^{-1}$ and kaolinite surface hydroxyl groups at $\sim 3400\text{ cm}^{-1}$) (Artz *et al*, 2008).

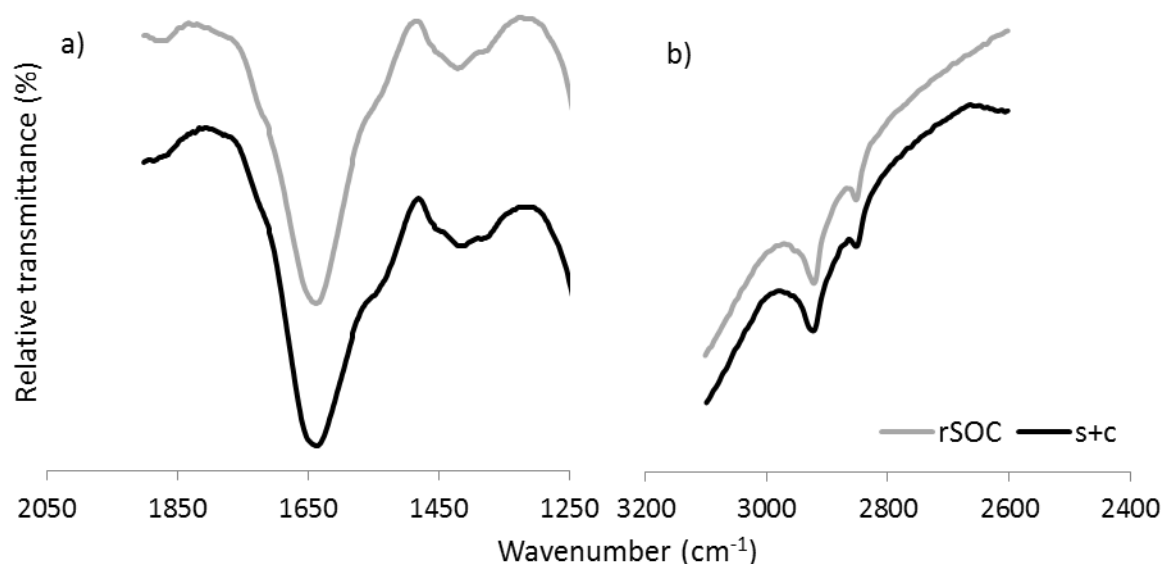


Figure 4.7: FT-IR spectra of s+c and rSOC fractions showing a) aliphatic carboxylates and/or aromatic group bands, b) aliphatic C-H stretching bands.

UV-vis Absorption Spectra Ratios of Liquid Fractions

The HWEC fraction contained more than 10 times more OC than the WSC (Table 4.3), although the WSC fraction had a $SUVA_{254}$ value of $7.81 \pm 1.67 \text{ L mg C}^{-1} \text{ m}^{-1}$, indicating a significantly higher aromatic content than the HWEC fraction ($0.52 \pm 0.12 \text{ L mg C}^{-1} \text{ m}^{-1}$) (one-way ANOVA, $p < 0.05$), consistent with results found by Xu *et al* (2012) for a forest soil. These results are in line with the longer MRT and lower ^{14}C content of the WSC fraction.

The DOC fraction $SUVA_{254}$ value was within the same range as WSC, indicating a similar level of aromaticity despite the much lower ^{14}C content in the DOC fraction. The E_4/E_6 ratio was significantly higher in the DOC fraction, suggesting that the OM in this fraction has a lower molecular weight than that of the Ghani fractions. However, E_4/E_6 ratios are sensitive to OC concentration and so the HWEC fraction cannot accurately be compared to the WSC or DOC fractions; removing the HWEC fraction from the analysis leaves no significant difference between the E_4/E_6 ratios for the WSC and DOC fractions.

Table 4.3: Specific UV absorbance at 245 nm (SUVA₂₅₄), E₄/E₆ ratios and organic C (OC) concentrations for water soluble C (WSC) and hot-water extractable C (HWE) isolated using the Ghani *et al* (2003) method and dissolved OC (DOC) isolated during the Zimmerman *et al* (2007) method. Standard errors are shown in parenthesis. Values with no common letters in each row are significantly different (One-way ANOVA).

	Ghani <i>et al</i>		Zimmermann <i>et al</i>
	WSC	HWE	DOC
SUVA ₂₄₅	7.81 (1.67) ^a	0.52 (0.12) ^b	10.36 (2.61) ^a
E ₄ /E ₆	0.68 (0.16) ^a	0.78 (0.20) ^a	1.59 (0.15) ^b
OC Concentration (mg l ⁻¹)	6.80 (0.44) ^a	70.67 (5.22) ^b	6.03 (0.94) ^a

Thermogravimetric analysis, hydropyrolysis and quality assurance tests

The TGA was conducted prior to AMS on a whole soil sub-sample to screen for black C contamination. No distinctive peak was observed between 450 and 590 °C (Leifeld, 2007) to suggest that black C was present, although small amounts may have been undetected.

After AMS analysis revealed the unusual ¹⁴C contents of the labile free LF, intra-aggregate LF and POM fractions, mass balance calculations of fraction ¹⁴C contents from both Zimmermann *et al* (2007) and Sohi *et al* (2001) fractionation methods were undertaken and found to be consistent with whole soil ¹⁴C contents. It was deemed possible that black C contamination could have resulted during contact with the density separation medium (NaI or SPT) which is recycled using activated C. Unfortunately, the feedstock for the activated C used could not be determined and so it was not possible to determine if this could be a source of very old C. Therefore, whole soil and POM fraction samples were screened for black C by HyPy analysis. Contact with the density separation medium would not explain the unusually low ¹⁴C content of the DOC, which is isolated before the soil comes into contact with the SPT. Never the less, ‘fresh’ (previously unused and therefore not recycled) SPT was prepared to eliminate this possibility when isolating POM for HyPy analysis.

The HyPy results found that the whole soil and POM sub-samples had estimated black C contents of ~5% (~1.7 t black C ha⁻¹) and ~17% (~0.4 t black C ha⁻¹), respectively, based on only one replicate for each. Brodowski *et al* (2006) quantified the proportion of C in density fractions (free POM with a density < 1.6 g cm⁻³ and occluded POM, released after shaking with glass beads, density < 1.6 g cm⁻³) composed of black C for a grassland site. The whole soil C (< 2 mm) was ~3% composed of black C and the combined free POM and occluded POM were ~7% of the combined fraction C (free POM + occluded POM) (Brodowski *et al*, 2006). However, this site was in a rural area of Germany; black C proportions of ~12 to ~13% of whole soil C and ~31 to ~42% of density fractions < 2 g cm⁻³ (inter- and intra-aggregate) were found for industrialised areas of Germany (Brodowski *et al*, 2007).

Camberdella and Elliott (1992) found that the POM of a grassland soil was composed of 5–10% black C by mass.

Quality assurance tests were undertaken to ensure that the black C was not unintentionally introduced to fractions as a consequence of laboratory conditions, equipment used or during the fractionation procedure itself. ^{14}C standards - barley mash samples of known ^{14}C age and an anthracite sample (^{14}C dead) - were exposed to all fractionation chemicals (except NaOCl) and equipment and then ^{14}C dated using AMS to assess whether any contamination with ^{14}C depleted material had occurred. These standards were found to be more *enriched* in ^{14}C than expected; suggesting modern material had been introduced during exposure to the fractionation conditions. Additionally, the residue from the whole soil and POM fraction HyPy analysis was also ^{14}C dated to determine the possible sources of the black C (i.e. fossil C or younger fire or pyrolysis derived black C). The residue was found to have a ^{14}C age of ~18000 years BP, confirming that the black C was derived from fossil material.

4.4 Discussion

4.4.1 Black C in SOM fractions

The high C:N and low ^{14}C contents of POM, free LF and intra-aggregate LF, and the low ^{14}C content of DOC, suggests these fractions may contain black C deposits from fossil fuel combustion (Lehndroff *et al*, 2015). The particulate nature of black C means that it is often located in the light SOM fractions (Glaser *et al*, 2000), and has been found to be preferentially located within light and aggregate-occluded density fractions of both grassland and forest soils (Brodowski *et al*, 2006). Black C has also previously been reported in soil DOC (Hockaday *et al*, 2006). This is consistent with the fractions from the Sohi *et al* (2001) and Zimmermann *et al* (2007) methods in which the black C was observed in this study, and also why a similar ^{14}C age was not recorded for either of the Ghani fractions which are extracted using a gentle procedure which does not break apart the stable aggregates.

Particles of black C have also been found associated with soil minerals, both large black C particles with small mineral particles and vice versa, through interaction with oxidised functional groups on the outer surface of the black C particles (Brodowski *et al*, 2005; Czimczik and Masiello, 2007). However, the ^{14}C contents and FT-IR spectra for the mineral fractions (organo-mineral, S+A, s+c and rSOC fractions) do not indicate that there was any black C associated with minerals in this soil.

Soils with black C contamination hamper the study of soil C dynamics using ^{14}C dating (Rethemeyer *et al*, 2004) and cause SOC dynamics models to underestimate turnover

(Lehmann *et al*, 2008). To overcome this, it could have been possible to model MRTs for this study by measuring the quantity of black C in each fraction and allocating this portion to the IOM pool of the RothC model (Rethemeyer, *et al*, 2007; Lehmann *et al*, 2008), however, HyPy was only carried out on the POM fraction and so this alternative approach was not possible.

4.4.2 Comparison of fractionation methods

Different soil fractionation methods isolate fractions which are essentially different mixtures of SOM and as such it is not possible to directly compare fractions which are defined by different parameters (i.e. different sizes, densities, resistance to chemical treatment). However, some comparisons can be drawn between similar fractions isolated using different methods.

The parameters which define the Zimmermann POM fraction during fractionation encompass those of both the Sohi free and intra-aggregate LFs combined (density $< 1.8 \text{ g cm}^{-3}$, between and within aggregates, respectively) with the added constraint that the POM fraction has a size $> 63 \text{ }\mu\text{m}$ (see Section 3.2). Despite this additional constraint, when the characteristics of the free and intra-aggregate LF fractions are combined, they make up a similar proportion of the whole soil C as the POM fraction (8.5 ± 0.4 and $9.7 \pm 1.2\%$, respectively), and although these two fractions have significantly different ^{14}C contents, when the ^{14}C contents are averaged the result is very similar to that of the POM fraction (62.3 ± 9.2 and $59.6 \pm 1.1 \text{ pMC}$, respectively). This suggests that the POM fraction is composed of a mixture of OM which could be further separated into two pools (inter- and intra-aggregate POM) which have distinct ages and chemical compositions (see Figures 4.4a and 4.4b).

Determining the age of SOM using ^{14}C dating depends on each isolated SOM fraction having homogenous, but distinctly different turnover rates from each other (von Lützow *et al*, 2007). From the findings in this study, it would appear to be more useful to use the Sohi *et al* fractionation method to isolate inter- and intra-aggregate ‘light’ fractions and treat these as separate pools for the purpose of modelling MRTs. However, the Sohi organo-mineral fraction contains all of the ‘heavy’ soil particles, including silt, clay and sand. Due to the low surface area of sand particles they have only a weak affinity for SOM (Christensen, 2001), and so this fraction has a relatively fast turnover, whilst SOM adsorbed to silt and clay is physico-chemically protected and is expected to have a longer turnover (von Lützow *et al*, 2007). Despite the Zimmermann S+A, s+c and rSOC fractions having similar ^{14}C contents in this soil, their abundance and C concentrations are important in terms of identifying and studying the relative significance of different SOM stability mechanisms and the impacts of land management practices on chemical and physical protection.

It is unfortunate that the presence of black C in the light fractions and DOC prevent the comparison of these fractions with the Ghani extracts (WSC and HWEC). The HWEC has been suggested as a sensitive indicator of the vulnerability of the labile SOM fractions to decomposition (Landgraf *et al*, 2006). It would have been useful to relate the turnover and chemical characteristics of these quickly and relatively cheaply isolated fractions to the labile Zimmermann POM and DOC and Sohi free LF to add evidence to support (or refute) their use as an indicator fraction.

4.4.3 Use of fractionation methods in the literature

In light of the results of this study it is pertinent to review previous studies which have used either the Sohi *et al* or Zimmermann *et al* methods to understand whether the correct choice of fractionation method was used. After consideration of a wide variety of studies using these methods, it was judged that in the majority of cases the fractionation method chosen was suitable for the aims of the study. However, there were a small number of cases where the Sohi *et al* method was used when it may have been more informative to use the Zimmermann *et al* method. Two examples are outlined below.

Freixo *et al* (2002) adapted the Sohi *et al* method to isolate two size fractions from the organo-mineral fraction (silt sized and clay sized fractions). The aims of the Freixo *et al* (2002) study were to determine how tillage and crop rotation affected the sensitivity of SOC fractions with different levels of stability. In this study it may have been better to use the Zimmermann *et al* method which more fully distinguishes between different SOC stabilisation mechanisms and perhaps modifying it to account for both free LF and intra-aggregate LF.

Shrumpf *et al* (2013) conducted a study to explore the factors controlling free LF and organo-mineral complexes in soils from 12 sites. They used the Sohi *et al* method; however radiocarbon dating of the isolated fractions showed no significant difference between the intra-aggregate LF and the organo-mineral fractions, whereas in the present study all of the Sohi *et al* fractions were significantly different. Shrumpf *et al* (2013) may have found more nuanced differences between fractions, however the aim of the study was to investigate stabilisation on minerals more generally and the cost of ^{14}C analysis of all whole soil and isolated fraction samples may have been restrictive.

In general, authors have adapted classic fractionation methods or developed new methods to fit the purpose of the particular study. This leads to interesting and important results, however our understanding of SOC stabilisation mechanisms would improve if universal methods could be agreed upon allowing comparisons to be drawn easily between studies with similar aims.

4.4.4 Relation to experiments in Chapters 5 and 6

The results of this fractionation methods comparison were used to inform the choice of fractionation methods used in Chapters 5 and 6 in this thesis. The purpose of the experiment in Chapter 5 '*South Road – reversion of soil carbon stocks after historic tillage treatments*' was to examine changes in C stabilisation mechanisms after land-use change. The Zimmermann *et al* method was deemed to be the most appropriate as it more fully segregates SOM fractions with different protection mechanisms. However, for the purposes of rapidly determining which soil depths to prioritise for fractionation work the Ghani *et al* method was employed to identify which depths were the most sensitive to land-use change.

The main purpose of Chapter 6 '*Soil fractions and respiration – direct assessment of physical protection and its influence on temperature sensitivity*' was to assess how the removal of physical protection (occlusion within aggregates) affects the susceptibility of OM to microbial decomposition at different temperatures. The Sohi *et al* method was deemed to be most appropriate in this case as it isolates the physically protected intra-aggregate labile fraction separately, and additionally the three fractions isolated using this method had distinctly different ^{14}C ages (and presumably MRTs).

4.5 Conclusions

This experiment was intended to answer three questions as set out in the introduction. However, due to the presence of black C contamination in the soil and isolated SOM fractions it was not possible to fully answer any of these questions, but I return to the questions here and provide a summary of the related findings.

1. *'For modellable SOM fractions isolated from the 0–10 cm layer of a managed grassland soil, what are the measured ^{14}C ages and MRTs and what do they mean in terms of C stability?'*

The conventional ^{14}C ages suggested that the presumed labile (containing young C) light fractions and DOC were much older than the mineral bound fractions. FT-IR analysis of all fractions and HyPy analysis of whole soil and POM samples suggest that this was due to black C contamination in these fractions. The chosen method of modelling MRTs was ineffective for these fractions but returned MRTs within expected ranges for the whole soil and mineral bound fractions. Therefore, it is difficult to assess the true level of stability of the labile fractions or relate this to the MRTs of the mineral bound fractions.

2. *'How do the ^{14}C ages and MRTs compare between fractions isolated using different fractionation techniques?'*

It was possible to draw some parallels between the light fractions from the Zimmermann *et al* (2007) and Sohi *et al* (2001) methods. However, it was difficult to compare the Sohi organo-mineral fraction with the Zimmermann S+A, s+c and rSOC fractions which had quite different MRTs from each other.

3. *'Do 'modellable' labile SOM/DOC fractions isolated from the 0–10 cm soil layer have similar MRTs to the labile SOC fractions (in the form of DOC) isolated using the simpler and cheaper hot-water extraction technique?'*

Unfortunately, it was not possible to draw any conclusions about the suitability of the HWEC fraction as an indicator fraction for labile soil C dynamics due to the black C contamination in the Sohi and Zimmermann labile fractions.

However, the experiment proved useful in informing the choice of fractionation method used in the following two chapters of this thesis.

5 South Road – reversion of soil carbon stocks after historic tillage treatments

5.1 Introduction

It is well established that cultivation of land leads to degradation of the soil organic C (SOC) stocks compared to the undisturbed state and that converting arable land to grassland can allow SOC stocks to recover, to an extent, over a number of decades (Burke *et al*, 1995; O'Brien and Jastrow, 2013). Rees *et al* (2005) estimated that 1.2–1.7 t C ha⁻¹ yr⁻¹ could potentially be sequestered by converting cropland to grassland in Europe; the IPCC (IPCC, 2000) estimate a global 50 year average sequestration of 0.8 t C ha⁻¹ yr⁻¹ after cropland to grassland conversion; Poeplau *et al* (2011) modelled SOC stock increases of ~40% and ~128% 20 and 100 years, respectively, after land-use change.

It has been demonstrated in numerous studies that tillage destabilises soil aggregates (e.g. Six *et al*, 1998; Grandy and Robertson, 2006; Kasper *et al*, 2009), thus reducing the opportunity for SOC to be physically and chemically protected in the soil matrix (Paustian *et al*, 2000). However, land management practices do not affect all parts of soil equally and the sensitivity of different soil organic matter (SOM) fractions may differ (Zimmermann *et al*, 2007c). While many studies report the reversion in SOC stocks after land use change from agriculture to grassland, the change in SOC stability within the soil matrix has rarely been directly measured (Six *et al*, 1998; Don *et al*, 2009; O'Brien and Jastrow, 2013; Poeplau and Don, 2013). John *et al* (2005) concluded that studying changes in SOC stocks should be accompanied by an assessment of C stability within the soil matrix to provide a better understanding of the changes in C dynamics and the function of SOM pools.

Changes in SOC stocks after land-use change usually take place very slowly (Simonsson *et al*, 2014), and so the majority of studies which record the change in SOC stocks after land-use change use paired plots, which are spatially close together but have been under different management for a known length of time (Poeplau and Don, 2013), or chronosequence studies where adjoining land has undergone the same land-use change but at different times (O'Brien and Jastrow, 2013). The problem with studies which substitute space for time is that they have inherent uncertainties associated with the spatial heterogeneity of soil and potential differences in the physical, chemical and climatic conditions of the plots prior to the divergence in land use, (Poeplau and Don, 2013). Few long-term studies of changes in SOC stocks after land use change exist (Six *et al*, 1998).

In this study the site of a long-term tillage treatment experiment, which ran from 1968 to 1991, was resampled in 2014. The main aim of this experiment was to determine how the

stability of the SOC had changed compared to archived soil sampled in 1975, seven years after the experiment was established. It was hypothesised that the physically unprotected particulate organic matter (POM) and dissolved organic C (DOC) fractions would be more sensitive than the mineral bound silt and clay (s+c) and sand and stable aggregate (S+A) fractions, and the chemically resistant (rSOC) fraction would be least sensitive to land-use change. The abandonment of this long-term tillage experiment provides a unique opportunity to assess the reversion of SOC stocks and changes in C stability from four different cultivation management practices.

5.2 Methods

5.2.1 Soil and tillage treatments

The South Road long term tillage treatment experiment began in 1967, examining crop and soil responses to contrasting tillage treatments for continuous spring barley production. The tillage treatments were applied to 12 x 48 m plots as described in Ball *et al* (1996):

- Deep plough (DP): inversion tillage to 35 cm from 1968–1983, then short term direct drill from 1983–1991.
- Shallow plough (SP): inversion tillage to 20 cm from 1968–1991.
- Chisel plough (CP): non-inversion tillage to 20 cm from 1968–1983, then broadcast and rotovation to 5 cm from 1983–1991.
- Long term direct drill (DD): seeds sown direct with no ploughing from 1968–1991.

There were eight replicates of each tillage treatment, four located on an imperfectly drained Eutric Cambisol of Macmerry series (loam topsoil, sandy clay loam subsoil) and four on a poorly drained Gleysol which is a Winton/Macmerry complex (loam to sandy clay loam topsoil, clay loam subsoil) (Ball *et al*, 1989) (see Figure 5.1). The field was sown with spring barley from 1968–1983, when it underwent a management change (as outlined above), after which it was sown with winter barley until 1991, except 1989 when the crop was oil-seed rape. The experiment was ceased in 1992. In 1996 the field was completely ploughed (to an unknown depth) and sown with grass until autumn 2013 when the field was ploughed (to 30 cm) and sown with winter wheat. The site has an annual average rainfall of 155 mm and an annual average temperature of ~10 °C (averaged from 1981 to 2010, Met Office, 2016).

Archived soils sampled in 1975 from all plots down to 36 cm depth (in 6 cm increments) were available. These samples had been collected using a spade and replicate plots for each treatment on each soil were bulked by depth to give a composite sample for each soil-treatment-depth combination. The soil was sieved to < 4 mm, air dried and sealed in plastic bags and placed in tins. These tins were stored in a cool dark shed until they were retrieved in 2014.

Samples were collected from the Gleysol (Winton/Macmerry series) in August 2014. The plots were relocated using detailed experimental field plans (Pidgeon, 1980) and aerial photographs of the site. Soil samples were taken in autumn 2014 after a second crop of winter wheat had been harvested. Soil was collected using an auger, and then split into 6 cm depth increments using a ruler and a sharp knife. As the soil was quite dry at the time of sampling in 2014, any cores which were not intact when extracted by the auger were discarded. For consistency, the replicate treatment plots were bulked by depth to give composite samples. The soil samples collected in 2014 had an average pH of 6.3.



Figure 5.1: Aerial view of the South Road former tillage treatment plots. Eutric Cambisol of Macmerry series marked in blue and Gleysol Winton/Macmerry complex marked in red.

This experiment assessed soils sampled from the Winton/Macmerry complex only (Figure 5.1). A detailed soil survey map was available (Pidgeon, 1980), and care was taken to sample from the Winton soil only, as had been done during the collection of the archived soils. In 2015, natural unmanaged Winton soil samples were collected from the fence edge, adjacent to an area of woodland to serve as a natural, unmanaged control soil for comparison with treated soils. Fence edge soil bulk density cores were collected at 0-6 cm, 18-24 and 30-36 cm and samples for CN analysis and fractionation were collected from each of these depths using a spade.

5.2.2 Effect of long term soil storage

To ascertain whether there was a significant loss of SOM during long term storage, archived 1975 soil was analysed by loss on ignition and loss on pre-treatment with hydrogen peroxide. Results were compared to measurements of OM made when the soil was initially sampled from the field in 1975.

Loss on ignition was determined by placing 10 g of air dried soil (sieved to < 4 mm) into pre-weighed tin cups. Tin cups were placed in a 105 °C oven to remove any moisture,

reweighed and then placed in a muffle furnace where the temperature was ramped up gradually to 550 °C and left for six hours. Samples were then re-weighed and OM concentration calculated from the loss in mass.

Loss on pre-treatment was determined for the DD and SP soil at 0–6 cm, 18–24 cm and 30–36 cm only. There was only one replicate of each sample and two blank (no soil) controls. Air dried soil (30 g, sieved to < 4 mm) was placed into a pre-weighed, tall 750 ml glass beaker and 30 ml of deionised water was added, followed by 250 ml of H₂O₂ (30%) (VWR, Poole, UK). The samples were thoroughly mixed and left to stand in a fume cupboard for 18 hours. The beakers were then gently heated on a hot plate with watch glasses placed on top to prevent evaporation. Drops of propylene glycol were added to the samples as an antifoaming agent to prevent frothing over the top of the beakers as the oxidation reaction occurred.

When the samples stopped gassing the watch glasses were removed and the solution was allowed to evaporate off, the samples were then left to cool slightly before a further 50 ml of H₂O₂ was added and then gently heated again. This process was repeated until there was no more gassing after addition of H₂O₂. Samples were then dried in a 105 °C oven and re-weighed.

5.2.3 SOC stocks

Whole soil was analysed for C and N concentrations using a Flash elemental analyser. Bulk densities were determined for all plots and fence edge soil at each depth in 1975 and 2015. Soil bulk densities measured in 2015 were significantly lower than those measured in 1975. This causes a bias in the SOC stocks when calculated using the conventional ‘fixed depth’ method (bulk density multiplied by C concentration) as a comparison is being made between different masses of soil. Therefore calculated ‘fixed depth’ SOC stocks for 2014 are not directly comparable to those calculated for 1975. Many studies have used the equivalent soil mass (ESM) approach to compare soil C stocks based on the same soil mass per unit area. Ellert *et al* (2001) defined ESM as the reference soil mass per unit area chosen in a layer. The equivalent soil C mass is the C mass stored in an ESM. In this study, minimum ESM C stocks were calculated. Following Lee *et al* (2009), the dry mass of soil for the *i*th year (M_i , Mg ha⁻¹) was calculated:

$$M_i = BD_i * T_i * 10^4$$

where BD_i is the bulk density in year *i* (Mg m⁻³), T_i is the thickness of the *i*th layer (m) and 10^4 is a unit conversion factor (m⁻² ha⁻¹). The mass of C at each fixed depth in the *i*th year ($C_{i, \text{fixed}}$, kg C ha⁻¹) was calculated as follows:

$$C_{i, \text{fixed}} = \text{conc}_i * M_i$$

where conc_i is the C concentration of the i th layer (kg C Mg^{-1}). The adjustment to the soil dry mass of each soil layer in year i ($M_{i,\text{add}}$) was calculated as the difference between the 2014 actual mass for each layer ($M_{i,\text{equiv}}$) and the 1975 actual mass – thus there was no adjustment necessary for the 2014 masses:

$$M_{i,\text{add}} = M_{i,\text{equiv}} - M_i$$

These parameters were then used to calculate ESM C stocks ($C_{i,\text{equiv}}$, Mg C ha^{-1}):

$$C_{i,\text{equiv}} = C_{i,\text{fixed}} - \text{conc}_{\text{top}} * M_{i,\text{add}} + \text{conc}_{\text{bottom}} * (M_{i,\text{add}} - M_{i-1,\text{add}})$$

where conc_{top} and $\text{conc}_{\text{bottom}}$ are the C concentrations (kg C Mg^{-1}) in the soil layer being corrected and the layer below it, respectively.

5.2.4 Fractionation

Four replicate sub-samples from each treatment – depth – year combination were initially fractionated using the Ghani *et al* (2003) method (results are shown in Appendix 3). The water-soluble and hot-water extractable fractions were analysed for C concentration and SUVA₂₅₄ and E_4/E_6 ratios were determined (see Section 3.2.3 for methodology). Analysis of these results suggested that the greatest differences in C storage would be found at the following depths: 0–6 cm, 18–24 cm and 30–36 cm. Based on this information, fractionations were only carried out on samples from these depths. Three replicate sub-samples from each of these depths for each treatment and year were then fractionated using the Zimmermann *et al* (2007) method (see Section 3.2.2).

5.2.5 Chemical analysis of fractions

Dissolved organic C (DOC) fractions were analysed for C concentration and SUVA₂₅₄ and E_4/E_6 ratios were determined (see Section 4.2.3 for methodology and calculations). All whole soil and solid fraction samples were analysed for C and N concentrations using a Flash elemental analyser and selected samples were also analysed by FT-IR spectroscopy (see Section 4.2.3 for methodology).

5.2.6 Statistical analyses

All statistical analyses were carried out in Genstat (Version 15.1). Within year variability was determined by two-way ANOVA and variability between years was determined by paired t-test. Significant differences were determined at $p < 0.05$. Post-hoc multiple comparison tests were applied to distinguish between means of different treatments. The sensitivity of isolated SOM fractions to land-use change was assessed by plotting the relative change (%) in the SOC stock stored in each fraction (from each sample) against the relative change in the ESM SOC stock between 1975 and 2014 and linear regression analysis was

used in Genstat to assess the correlation between these two values (after Poeplau and Don, 2013).

5.3 Results

5.3.1 Loss of organic matter during long term storage

Loss on ignition and loss on pre-treatment with H₂O₂ of soils sampled in 1975 and measured in 2015, after 40 years of storage, were consistently higher than values of OM measured in 1975 (Table 5.1). Differences are likely to be due to improved measurement efficiency (e.g. better muffle furnace operating conditions and the use of fresher H₂O₂ during the 2015 measurements) as it is unlikely that the archived soil could have gained organic C during storage.

Estimating % OM contents from % OC contents of stored soil sampled in 1975 and measured in 2015, by multiplying by a conversion factor of 1.724, gave OM content values 90–120% of those measured in 1975. The exception was DP 30–36 cm which was only 74% of the 1975 value (see Table 5.1).

Table 5.1: Mean values of organic matter (OM) contents measured for the same archived soil samples in 1975 and 2015. Loss on pre-treatment was carried out using hydrogen peroxide and OM was estimated by applying a conversion factor of 1.724 to measured C concentrations. LOI – loss on ignition, TC – total C and nd – not determined.

Treatment	Depth (cm)	Measured 1975 (mean % w/w)		Measured or estimated 2015 (mean % w/w)		
		loss on pre-treatment	OM content	loss on pre-treatment	LOI	Estimated OM content*
CP	0–6	3.0	5.79	5.89	7.48	5.72
	18–24	2.7	5.02	5.20	6.95	5.23
	30–36	1.4	2.00	3.00	4.54	1.89
DD	0–6	3.7	5.69	5.91	6.17	6.98
	18–24	2.3	3.30	4.66	5.71	3.62
DP	0–6	2.8	4.41	nd	7.35	4.46
	18–24	3.3	4.72	nd	6.07	4.52
	30–36	2.4	4.35	nd	5.46	3.23
SP	0 - 6	3.7	5.25	nd	7.28	5.87
	18 - 24	4.0	6.18	nd	6.28	5.68
	30 - 36	2.8	5.25	nd	6.32	4.83

* Estimated from measured OC content (%)

Loss on ignition was also determined for 0 – 15 cm samples from these plots in 1988 with average values of 7.3, 6.3, 7.0 and 7.4% w/w for DD, DP, SP and CP, respectively (Ball *et al*, 1994). These values are within the range of those measured from archived 0–6 cm samples in 2015, with only the DD treatment being distinctly lower. However, % TC

was also measured for 1988 DD 0-6 cm samples (Ball *et al.*, 1996), and converting these into % OM gave a value of ~7% w/w.

Although none of these values are directly comparable, and therefore it is impossible to state definitively that there was no OM loss during storage, it seems reasonable to conclude that previously and recently measured OM contents for archived soils are similar enough to allow any residual effects from tillage treatments to be detected, if not accurately quantified.

5.3.2 SOC stocks

Across the whole profile, there were increases in mass corrected SOC stocks from 1975 to 2014 for all treatments except SP which had a reduction (18, 37, 13 and -15% changes for CP, DD, DP and SP, respectively) (Figure 5.2 and Table 5.2). In the upper 24 cm, all treatments appeared to follow a similar gradient of SOC change, except the DP treatment which had a 30% increase and had significantly higher SOC stocks than all other treatments from 6–12 cm in 2014. The more intensively (inversion) tilled plots (DP and SP) experienced SOC losses below 24 cm (average declines of ~27 and ~37% for DP and SP from 24–36 cm, respectively) whilst the less intensive (non-inversion) DD and CP treatment plots displayed average SOC increases of 47% between 24–36 cm from CP plots and 115% between 24–30 cm from the CP plots.

These differences in direction of change served to even out SOC stocks across the field, effectively obliterating any residual effects of the long term tillage treatments on C stocks. For example, at 24–30 cm the SP stocks declined by $27 \pm 5\%$ and the DP by $39 \pm 3\%$ between 1975 and 2014 but the stocks were considerably higher (39 ± 3 and 30 ± 2 kg C ha⁻¹, Table 5.2) than the DD and CP treatment (21 ± 0.6 and 15 ± 0.4 kg C ha⁻¹, respectively) in 1975. In 2014 the SOC stocks were in closer agreement (27 ± 1 , 26 ± 0.4 , 18 ± 0.3 and 28 ± 0.4 for CP, DD, DP and SP, respectively), although interestingly the SOC stock in the DP treatment plots declined to significantly lower values ($p < 0.001$) than the other treatments.

Equivalent soil mass SOC stocks for the fence edge woodland soil were calculated against each tillage treatment in 2014. The same could not be done for 1975 tillage treatment SOC stocks as no bulk density data was available for the fence edge soil in 1975. When compared to the 2014 tillage treatment plots SOC stocks (Table 5.2), the fence edge woodland soil OC stocks were consistently significantly higher ($p < 0.01$) than tillage treatment soils from 0–6 and 30–36 cm depth, but significantly lower than CP, DD and DP ($p < 0.01$) soils from 18–24 cm depth.

Table 5.2: Equivalent soil mass (ESM) soil organic C (SOC) stocks for chisel plough (CP), direct drill (DD), deep plough (DP) and shallow plough (SP) tillage treatment plots in 1975 and in 2014, 22 years after tillage treatments were ceased and ESM SOC stocks for fence edge woodland soil in 2015 (corrected against each tillage treatment for comparison) at three depths (fence edge woodland soil could not be corrected to 1975 ESM stocks as no bulk density data was available. Standard errors are in parenthesis. * denotes significantly higher ESM SOC stock between the 2014 and fence edge stocks.

Depth (cm)	1975 ESM SOC stocks (Mg C ha ⁻¹)							2014 ESM SOC stocks (Mg C ha ⁻¹)						
	0 - 6	6 - 12	12 - 18	18 - 24	24 - 30	30 - 36	0 - 36	0 - 6	6 - 12	12 - 18	18 - 24	24 - 30	30 - 36	0 - 36
CP	29.8 (0.2)	32.4 (0.4)	32.2 (0.7)	28.5 (0.4)	18.3 (0.5)	12.8 (0.3)	154	26.3 (0.4)	30.1 (0.3)	35.6 (0.8)	34.9 (1.0) *	26.9 (1.1)	19.0 (0.1)	172
DD	35.5 (2.1)	36.3 (0.7)	30.7 (0.3)	21.5 (1.1)	12.1 (0.3)	-	136	30.5 (0.5)	34.8 (0.1)	37.5 (0.4)	35.1 (1.7) *	26.0 (0.4)	16.4 (0.2)	164
DP	24.8 (0.4)	-	26.8 (0.2)	26.8 (1.0)	25.1 (1.6)	21.6 (0.9)	104	32.2 (0.2)	37.9 (0.2)	39.8 (0.5)	30.0 (1.0) *	18.4 (0.3)	15.6 (0.4)	158
SP	31.9 (0.8)	29.0 (0.5)	27.3 (0.2)	30.9 (0.8)	36.0 (2.8)	35.0 (0.5)	155	30.6 (1.1)	26.6 (0.9)	34.8 (1.8)	30.9 (0.5)	27.9 (0.4)	16.9 (0.1)	151
							CP	54.3 (0.7) *	-	-	25.8 (0.5)	-	32.8 (1.0) *	-
							DD	60.1 (0.8) *	-	-	22.1 (0.4)	-	26.3 (0.8) *	-
							DP	50.6 (0.7) *	-	-	22.6 (0.4)	-	27.7 (0.8) *	-
							SP	50.9 (0.7) *	-	-	32.8 (0.7)	-	29.3 (0.9) *	-

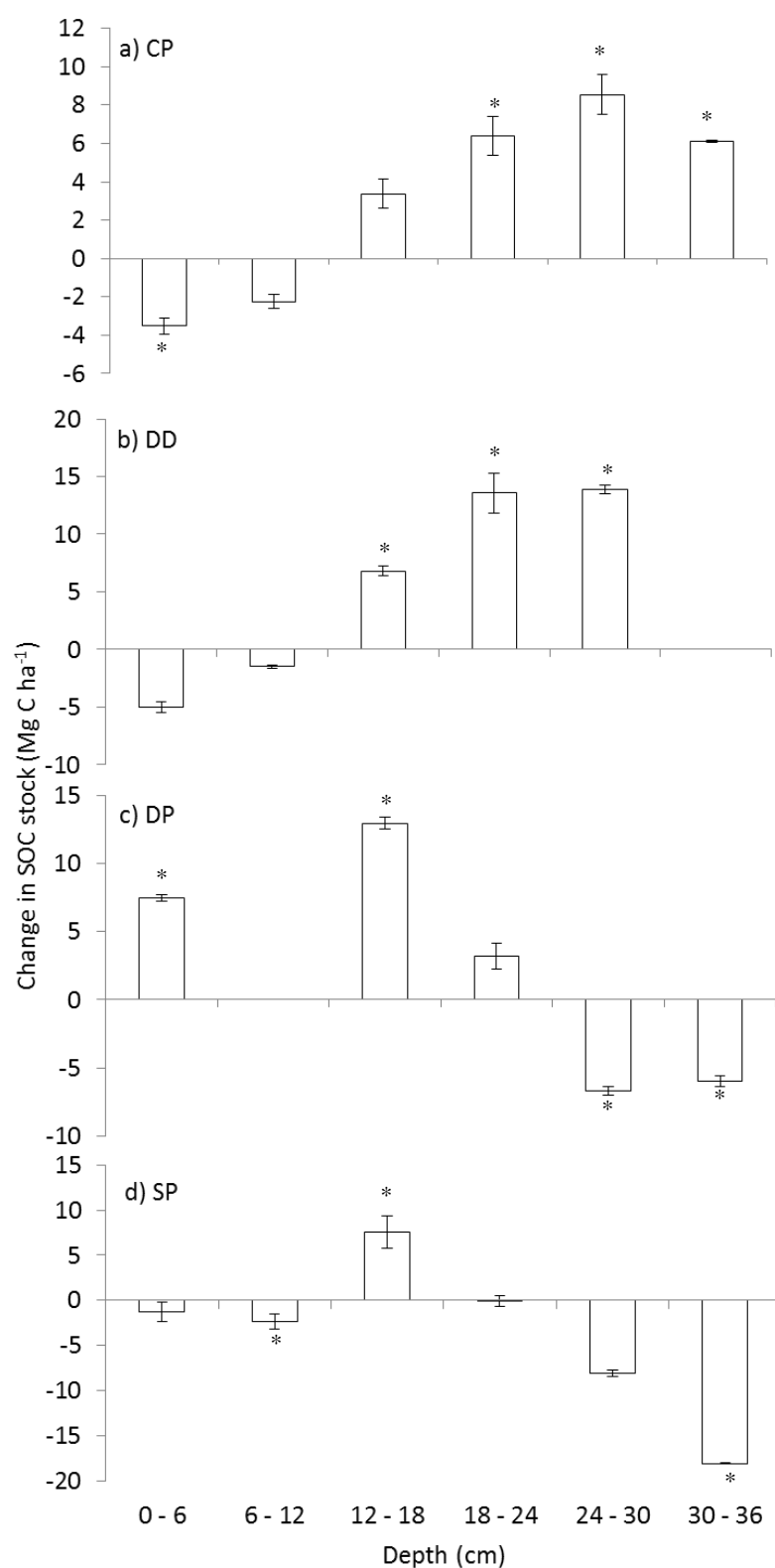


Figure 5.2: Change in soil organic C (SOC) stocks for tillage treatment plots (a) chisel plough (CP), b) direct drill (DD), c) deep plough (DP) and d) shallow plough (SP) from 0–36 cm (at 6 cm intervals) from 1975 and 2014, 22 years after tillage treatments had ceased. Note that there was no SOC stock data available for direct drill 30–36 cm or deep plough 6 – 12 cm. * denotes a significant change in SOC stock from 1975 or 2014 (paired t-test, $p < 0.05$).

5.3.3 SOM fractions as a proportion of whole soil mass

The efficiency of soil mass recovery during the fractionation process ranged from 94–101% of the original whole soil mass. Generally, there was greater variation in fraction proportions between treatments in 1975 than in 2014 (Figure 5.3, Table 5.3).

The relative mass of soil contained in the S+A fraction tended to be significantly higher ($p < 0.01$) at all depths in 2014 compared to 1975. Average increases across treatments were 3.7 ± 0.3 and $5.4 \pm 0.7\%$ from 0–6 and 18–24 cm, respectively. Changes in the proportions of S+A fraction mass from 30–36 cm were much more variable with increases of 9.3 ± 1.4 , 0.4 ± 1.7 and $2.8 \pm 0.4\%$ for CP, DP and SP treatments, respectively (note that no comparison could be made for the DD treatment at this depth). The fence edge woodland soil had significantly higher relative masses of POM for all treatments and depths in both years and significantly higher relative mass proportions of DOC from 0–6 and 30–36 cm in both years.

5.3.4 Distribution of SOC between SOM fractions

The efficiency of soil C recovery during the fractionation procedure ranged from 94–123% of the estimated original whole soil C for the 1975 samples and 73–119% for the 2014 samples. The wider variation in C recovery compared to soil mass recovery is likely due to the presence of coal fragments in the soil and variability in the concentration of coal between fractionated samples. To allow a statistical comparison between treatments and years, fraction C was analysed as a relative proportion of the recovered C rather than the absolute proportion calculated.

In terms of C stability, the proportion of SOC stock C contained within each fraction is a more important factor than the proportion of SOM mass that each fraction comprises. For example, the POM fraction makes up a very small proportion of the soil but is rich in C. It is also possible that the rSOC fraction could increase its C concentration significantly without necessarily increasing its mass greatly.

In contrast to the increase in mass proportions of S+A, there were significantly lower proportions of C stored in the S+A fraction of all treatments in 2014 compared to 1974 from 18–24 cm ($p < 0.01$) (6.4 ± 0.8 , 9.4 ± 2.5 , 3.5 ± 1.0 and $7.2 \pm 0.8\%$ decreases for the CP, DD, DP and SP treatments, respectively) (Figure 5.4); absolute values of fraction SOC stocks for each treatment and depth are in Appendix 3. Only the inversion plough treatments (DP and SP) had significantly lower proportions of SOC in the 2014 S+A fraction from 0–6 cm depth (3.9 ± 0.6 and $5.4 \pm 1.6\%$ decreases for the DP and SP treatments, respectively, $p < 0.05$). Proportions of SOC in the 2014 S+A fraction from 30–36 cm depth were not significantly different. All treatment and depth combinations had significantly

higher proportions of C stored in S+A compared to the fence edge woodland soil, perhaps due to C redistribution processes and soil structural changes within the agricultural soils. There were no other clear trends across treatments in the relative proportions of SOC stock C contained in any fractions. However, within the SP treatment from 30–36 cm there were significantly lower proportions of SOC stock C contained within the rSOC, POM and DOC fractions in 2014 (16.0 ± 1.6 , 22.6 ± 5.6 and $1.9 \pm 0.5\%$ decreases, respectively).



Figure 5.3: Percentage (by mass) of a) 1975 and b) 2014 whole soil made up by sand and stable aggregates (S+A), isolated silt and clay (s+c) less the residual soil organic C (rSOC), particulate organic matter (POM) and dissolved organic carbon (DOC) for different tillage treatments. CP – chisel plough, DD – direct drill, DP – deep plough, SP – shallow plough, F0, F18 and F30 – fence edge woodland soil 0–6 cm, 18–24 cm and 30–36 cm respectively.

Table 5.3: Significant differences (ANOVA, $p < 0.05$) between proportions of whole soil mass contained in different Zimmermann fractions from tillage treatments at soil depths of 0–6, 18–24 and 30–36 cm in 1975 and 2014. Treatments within each depth which have no common letters in a column are significantly different from each other (where there are no letters there were no significant differences between treatments at that depth). * denotes that the fraction had a significantly greater whole soil mass proportion than the corresponding fraction in the other year. S+A – sand and aggregates, s+c-rSOC – silt and clay less the residual soil organic C (rSOC), POM – particulate organic matter, DOC – dissolved OC.

Depth (cm)	Treatment	1975					2014				
		S+A	s+c-rSOC	rSOC	POM	DOC	S+A	s+c-rSOC	rSOC	POM	DOC
0–6	Chisel plough		*	b	a	a	ab*	a	b	a	a
	Direct drill			b	a	a	b*	a	b	a	b
	Deep plough			b	a	a	ab*	ab	b	a	ab
	Shallow plough			b	a	a	b*	ab	b	a	ab
	Fence edge woodland			a	b	b	a	b	a	b	c
18–24	Chisel plough	a	b	*	bc		*	a			
	Direct drill	ab	a		a		*	a			*
	Deep plough	a	ab		ab		*	b			
	Shallow plough	a	ab		ab		*	a			
	Fence edge woodland	b	a		c			b			
30–36	Chisel plough	a	b	b*	a	a	b*	a	a	a	a
	Direct drill	-	-	-	-	-	ab	ab	ab	ab	a
	Deep plough	b	ab	ab	b	ab	a	b	ab	a	a
	Shallow plough	c	a	a	b	ab	ab*	ab	ab	a	a
	Fence edge woodland	d	a	a	c	b	ab	ab	b	b	b

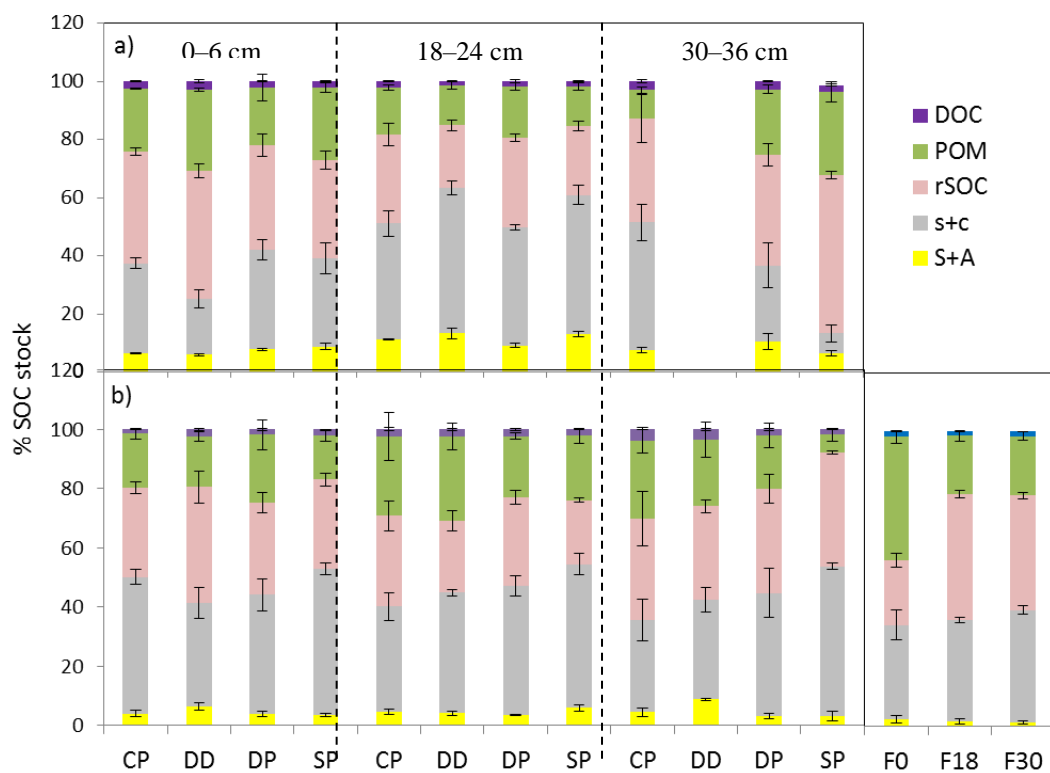


Figure 5.4: Relative proportions of soil organic C (SOC) stock (expressed as percentages) made up by sand and stable aggregate C (S+A), silt and clay C (s+c) less the residual soil organic C (rSOC), particulate organic matter C (POM) and dissolved organic C (DOC) for a) 1975 and b) 2014 soils. Note that there was no direct drill 30 – 36 cm sample in 1975. CP – chisel plough, DD – direct drill, DP – deep plough, SP – shallow plough, F0, F18 and F30 – fence edge 0–6 cm, 18–24 cm and 30–36 cm, respectively.

Table 5.4: Significant differences (ANOVA, $p < 0.05$) between proportions of soil organic C stocks contained in different Zimmermann fractions from tillage treatments at 0–6, 18–24 and 30–36 cm in 1975 and 2014. Treatments within each depth which have no common letters in a column are significantly different. 'ns' denotes that there were no significant differences. * denotes that the fraction had a significantly greater total soil C proportion than the corresponding fraction in the other year. S+A – sand and aggregates, s+c-rSOC – silt and clay less the residual soil organic C (rSOC), POM – particulate organic matter, DOC – dissolved OC.

Depth (cm)	Treatment	1975					2014				
		S+A	s+c-rSOC	rSOC	POM	DOC	S+A	s+c-rSOC	rSOC	POM	DOC
0 - 6	Chisel plough	c	b	b*	a	*	b	a*	d	a	a
	Direct drill	d	a	c	a*	ns	b	b	c	a	c
	Deep plough	bc*	b	bc	a	ns	c	b	ab	a	ab
	Shallow plough	ab*	b	b	a*	ns	d	b*	bc	a	bc
	Fence edge woodland	a	a	a	b	ns	a	a	a	b	a
18 - 24	Chisel plough	c*	b	b	b	ns	c	b	a	ns	b
	Direct drill	c*	c*	ab	b	ns	c	ab	a	*	ab*
	Deep plough	b*	c	b	ab	ns	b	ab	b	ns	b
	Shallow plough	d*	d	a	a	ns	c	a	ab	*	b
	Fence edge woodland	a	a	c	b	ns	a	a	c	ns	a
30 - 36	Chisel plough	d	a	b	a	b	bc	b	ab	*	c
	Direct drill	-	-	-	-	-	c	a	c	ns	b
	Deep plough	c	a	a	c	ab	b	c	a	ns	bc
	Shallow plough	b	b	a*	ab*	ab*	b	b*	ab	ns	b
	Fence edge woodland	a	a	b	bc	a	a	bc	b	ns	a

5.3.5 Whole soil and SOM fraction C:N

Whole soil C:N at different depths remained fairly stable between treatments in 1974 and 2014, although C:N were generally lower in 2014 than 1975 (Table 5.5).

The C:N of the S+A fractions (Table 5.6) showed a trend between years similar to that of the SOC distribution. The S+A fractions generally had significantly higher proportions of whole soil C and C:N in 1975. The s+c fractions generally had significantly higher C:N in 2014, suggesting the OM was more highly decomposed.

Statistical analyses of C:N within and between years for the rSOC fraction was made difficult as many samples had N concentration which were below the detection limit of the CN analyser and so calculation of C:N was limited. Where comparisons were possible, C:N were significantly higher in 2014 compared to 1975.

Table 5.5: Average C:N for whole soil samples from four tillage treatments in 1975 (8 years after treatments began) and 2014 (22 years after treatments ceased) at 6 cm depth intervals. Standard errors are given in parenthesis where they were ≥ 0.01 . Treatments with different letters beside values within a depth were significant different in that year (one-way ANOVA, $p < 0.05$). The 1975 values were compared to 2014 woodland values where appropriate. * denotes values which are significantly higher than the corresponding value in the other year (paired t-test, $p < 0.05$).

Chisel Plough		Average C:N	
Depth (cm)	Treatment	1975	2014
0–6	Chisel plough	14.59 (2.61)	16.11 (0.04)b
	Direct drill	17.11 (1.08)	15.15 (0.21)b
	Deep plough	18.29 (0.93)	16.03 (0.49)b
	Shallow plough	19.50 (0.57)*	10.74 (1.86)a
	Woodland	-	12.33 (0.07)ab
6–12	Chisel plough	17.84 (1.28)	15.15 (0.03)
	Direct drill	18.31 (0.59)*	15.31 (0.22)
	Deep plough	-	14.95 (0.67)
	Shallow plough	19.50 (0.58)	9.45 (0.89)
12–18	Chisel plough	17.73 (0.65)*	14.07 (0.09)
	Direct drill	19.42 (1.30)*	14.18 (0.21)
	Deep plough	17.20 (1.36)	14.44 (0.82)
	Shallow plough	19.47 (0.82)*	11.89 (0.79)
18–24	Chisel plough	18.25 (0.79)ab*	14.33 (0.14)
	Direct drill	19.42 (1.21)b*	14.13 (0.11)
	Deep plough	18.08 (0.84)ab	14.49 (2.36)
	Shallow plough	20.08 (1.38)b	12.49 (0.68)
	Woodland	- a	13.82 (0.27)
24–30	Chisel plough	18.09 (0.81)	16.32 (0.34)
	Direct drill	13.84 (4.15)	15.20 (0.14)
	Deep plough	18.97 (1.27)	16.86 (1.56)
	Shallow plough	17.88 (0.79)*	10.92 (0.28)
30–36	Chisel plough	19.42 (1.70)b	16.58 (0.10)
	Direct drill	-	15.38 (0.30)
	Deep plough	17.41 (1.11)ab	24.83 (3.63)
	Shallow plough	20.47 (1.01)b	16.85 (6.67)
	Woodland	- a	12.91 (0.13)

No statistical comparisons of POM fractions in 1975 or between years could be made as only one sample was measured for each treatment in 1975. The wide variation of C:N in the 2014 POM samples, most likely due to the presence of coal fragments which can have very high C:N, also make it difficult to speculate on any trends.

Table 5.6: Average C:N for Zimmermann soil organic matter fractions from four tillage treatments in 1975 (8 years after treatments began) and 2014 (22 years after treatments ceased) at 6 cm depth intervals. Standard errors are given in parenthesis where they were ≥ 0.01 . Treatments with different letters beside values within a depth were significant different in that year (one-way ANOVA, $p < 0.05$). The 1975 values were compared to 2014 fence edge woodland values where appropriate. * denotes values which are significantly higher than the corresponding value in the other year (paired t-test, $p < 0.05$). NA – no C:N could be calculated as the N concentration was lower than the detection limit of the CN analyser. S+A – sand and stable aggregates, s+c – silt and clay, rSOC – residual soil organic C, POM – particulate organic matter.

Depth (cm)	Treatment	1975				2014			
		S+A	s+c	rSOC [‡]	POM [†]	S+A	s+c	rSOC [‡]	POM [†]
0–6	Chisel plough	20.74 (0.11)ab *	11.22 (0.18)a	38.94 (0.48)a	70.26	9.84 (1.99)	14.60 (0.33) *	NA	37.56 (1.58)
	Direct drill	21.01 (0.93)ab *	11.23 (0.28)a	40.98 (1.27)a	60.42	11.30 (2.57)	12.00 (3.05)	NA	50.97 (14.99)
	Deep plough	18.72 (0.93)a *	11.87 (0.21)a	40.34 (0.46)a	49.14	9.37 (1.38)	14.31 (0.75) *	72.30 (0.46)a	99.17 (42.90)
	Shallow plough	11.48 (0.24)b *	11.48 (0.24)ab	43.25a	63.83	9.73 (1.80)	15.07 (0.13) *	123.6b	39.25 (2.86)
	Woodland	-	- b	- b	-	NA	16.73 (2.14)	97.47 (9.40)ab	18.45
18–24	Chisel plough	15.41 (0.51) *	11.11 (0.06)	41.75 (5.28)	39.84	9.31 (2.02)	14.87 (0.57) *	NA	125.4 (56.14)
	Direct drill	16.71 (0.90) *	11.17 (0.25)	36.27	46.62	6.85 (0.65)	14.78 (0.39) *	98.43 (5.97)a	144.8 (50.97)
	Deep plough	18.25 (1.18) *	11.64 (0.17)	42.16 (2.45)	56.34	7.77 (0.94)	13.88 (0.88)	104.0 (11.49)a *	32.33 (1.45)
	Shallow plough	11.36 (0.06) *	11.36 (0.06)	35.71 (0.63)	43.84	9.31 (2.02)	18.44 (3.84)	55.53 (1.00)a	43.73 (2.68)
	Woodland	-	-	-	-	NA	16.91 (4.64)	201.0 (104.45)b	38.51
30–36	Chisel plough	16.71 (1.31) *	10.4 (0.35)a	29.82 (6.16)a	122.63	5.11 (0.99)a	14.77 (0.86)a *	99.97 (1.43)bc *	44.8 (4.56)a
	Direct drill	-	-	-	-	21.61 (0.29)b	10.17 (0.45)a	0.01a	260.7b
	Deep plough	22.89 (3.26)	11.18 (0.38)a	35.02 (1.03)a	90.07	8.57 (4.03)ab	13.05 (2.22)a	52.08 (1.91)ab *	97.8 (45.02)a
	Shallow plough	11.05 (0.18)	11.08 (0.18)a	40.69 (0.26)a	87.52	5.11 (0.99)ab	16.09 (0.57)a *	55.20 (0.12)ab *	539.8 (17.88)c
	Woodland	-	- b	- b	-	NA	24.52 (1.77)b	133.5 (22.87)c	39.78a

[†] Where there is no standard error given for POM fractions only one sample was measured, thus no statistical analysis could be carried out within year for 1975 or between years.

[‡] Where there is no standard error given for rSOC fractions only one C:N ratio could be calculated as N concentrations were below the detection limit of the CN analyser for other replicates, thus no statistical analysis could be carried out within or between years for these treatments.

5.3.6 Chemical characterisation of fractions

Comparisons of FT-IR spectra for the CP POM fractions at 0–6 cm and 18–24 cm are shown in Figure 5.5 with the four major absorption bands (hydroxyl, aliphatic, aliphatic carboxylate/aromatic and polysaccharide/silicate groups) indicated. Peak assignments were according to Palacio *et al* (2014).

The broad absorption band around 3400 cm^{-1} , attributed to H bonded OH groups of OM and/or on mineral (e.g. kaolinite) surfaces, was well pronounced in all POM fractions but was slightly more intense in the 2014 spectra at 0 – 6 cm and much stronger in 2014 in the 18–24 cm layer. The wider bands are indicative of greater humification. The aliphatic C-H stretching groups (2920 and 2850 cm^{-1}) were more pronounced in the 1975 soil at 0–6 cm, but were stronger in the 2014 soil in the 18–24 cm layer. However, the aliphatic carboxylates and/or aromatic group bands (around 1610 cm^{-1}) remained strongest in the 2014 POM fractions at both depths.

Absorption bands around 1000 cm^{-1} indicated that C-O stretching of polysaccharide or polysaccharide-like substances (or possibly silicates present in the POM fractions) were stronger in 2014 for both depths but were particularly strong in the 2014 18–24 cm POM fraction, suggesting that the 2014 fractions may have been less humified. This is in line with an increase in C:N from 37 in 1975 to 70 in 2014 in the 0–6 cm layer; however, the 18–24 cm layer C:N decreased from 69 in 1975 to 40 in 2014. This unexpected result may be caused by the inversion tillage events which took place in 2013 and 2014.

There were no significant differences in SUVA_{254} values for DOC samples, either between treatments within years or between years (Figure 5.6). Values ranged from 1.8 ± 0.4 to $4.0 \pm 1.3\text{ L mg C}^{-1}\text{ m}^{-1}$.

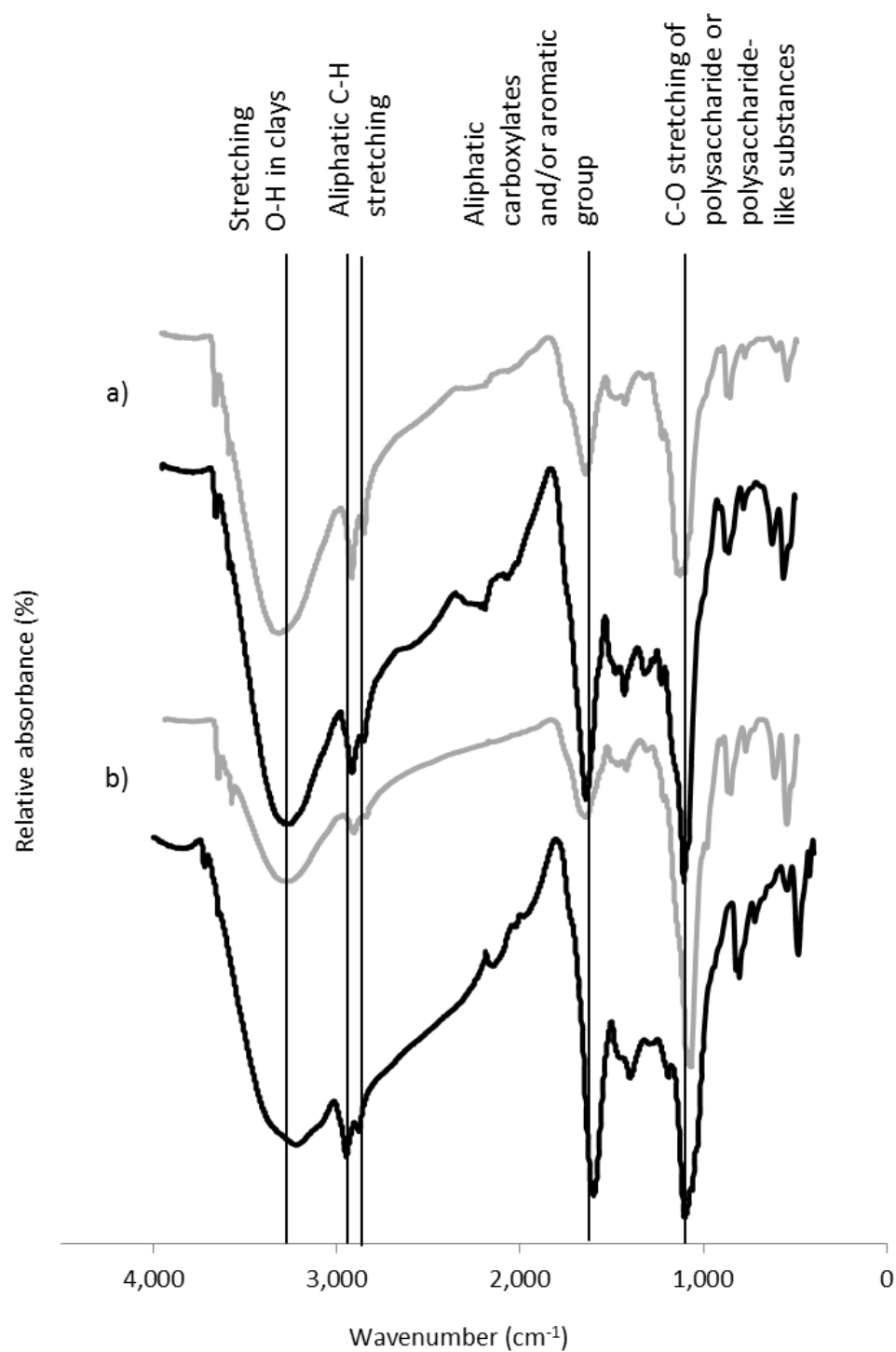


Figure 5.5: FT-IR spectra for the chisel plough (CP) particulate organic matter (POM) fractions from a) 0–6 cm and b) 18–24 cm in 1975 (grey lines) and 2014 (black lines).

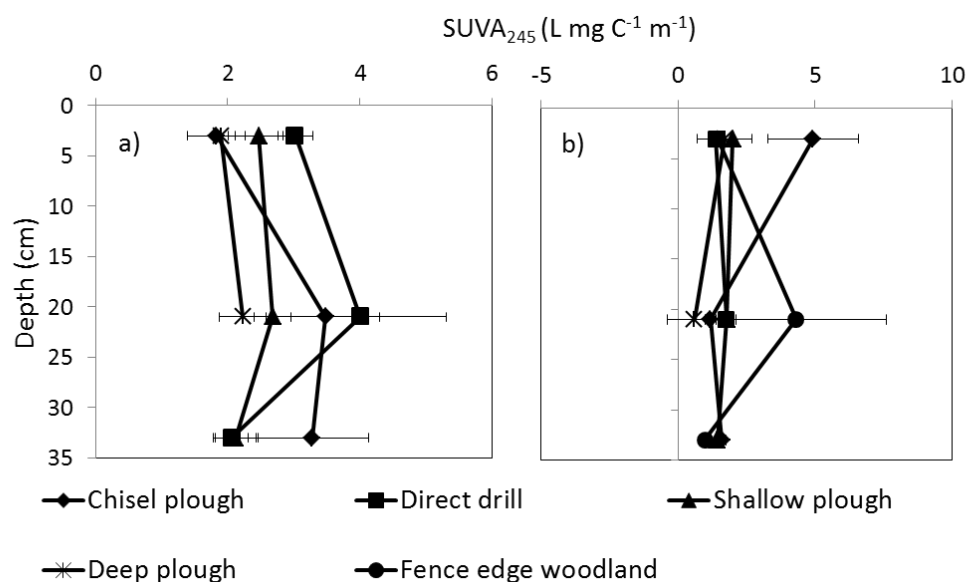


Figure 5.6: Specific UV Absorbance at 245 nm ($SUVA_{245}$) of dissolved organic C for chisel plough, direct drill, shallow plough and deep plough treatments in a) 1975 and b) 2014, 22 years after tillage treatments had ceased. Fence edge woodland soil was also analysed in 2014.

5.3.7 Sensitivity of SOC fractions to land use change

Relative (%) changes in ESM SOC stocks of fractions, rather than absolute values, were used to assess the sensitivity of SOM fractions to land-use change to allow a fair comparison between fractions which contain large (i.e. rSOC) and small (i.e. DOC) quantities of C. The sensitivity of the different pools to a change in land use from arable to grassland varied between treatments and depths, and changes were not consistently in the same direction between treatments.

The sensitivity of isolated SOM fractions followed the order $POM > DOC > rSOC$ for all treatments (see Figure 5.7 for plots and Table 5.7 for regression equations, R^2 values and significance levels). The labile POM and DOC fractions were most sensitive in all instances but were more sensitive in the lower impact CP and DD treatments (factors of 5.3 and 3.8 for POM and 2.3 and 2.8 for DOC in the CP and DP treatments, respectively, as indicated by the slope of the regression line) than in the inversion treatments DP (2.0 and 1.1 for POM and DOC, respectively) and SP (2.2 and 1.8 for POM and DOC, respectively).

In some cases the correlation between changes in ESM SOC stock and fraction SOC stock were not significant. In particular, the S+A fraction was not significant for any treatment. The s+c-rSOC fraction only had a significant correlation between total SOC stock change and fraction C stock change in the SP treatment ($p < 0.05$, $R^2 = 0.53$) (see Figure 5.7 and Table 5.7).

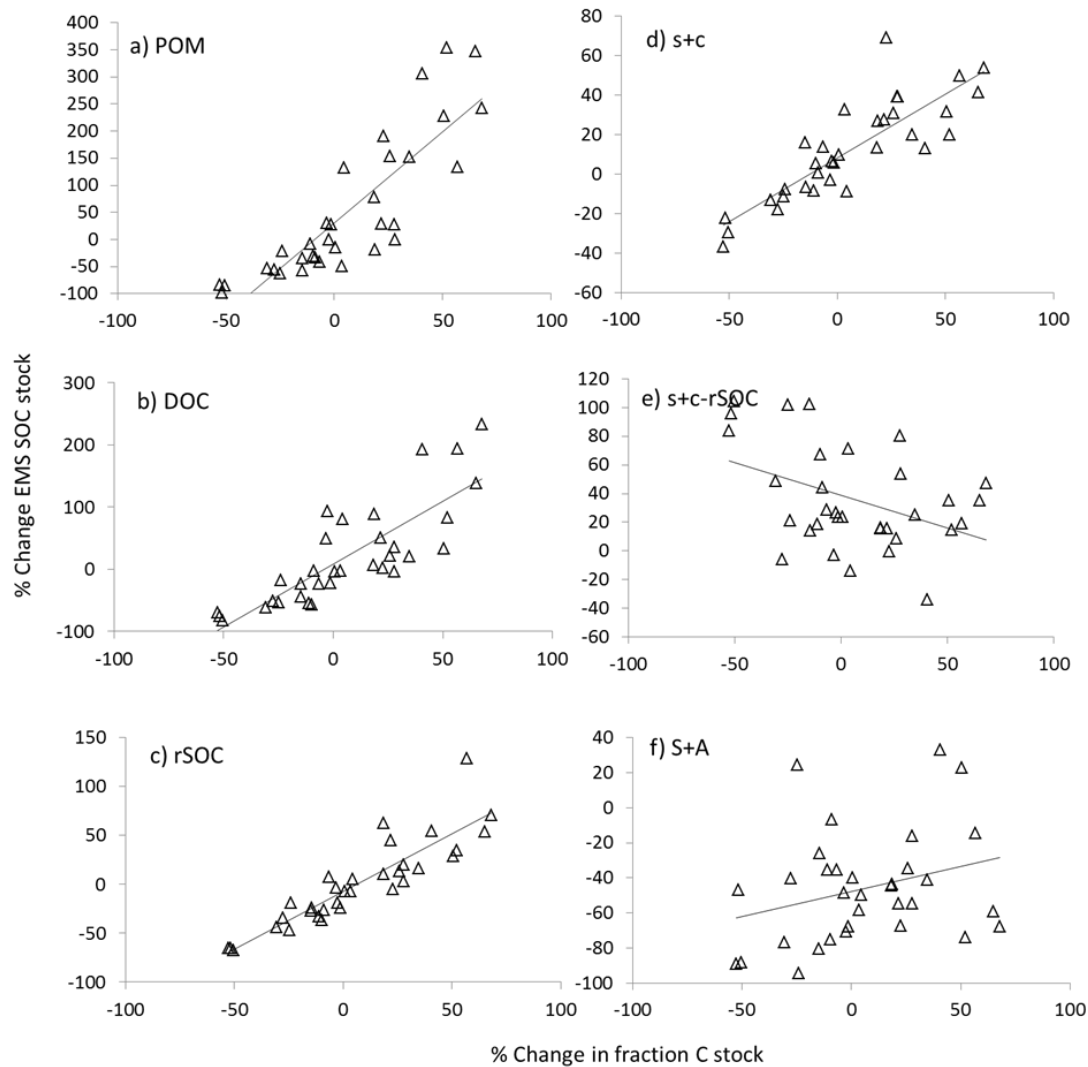


Figure 5.7: Regression plots for the relative (%) change in equivalent soil mass (ESM) soil organic C (SOC) stocks against the relative change in the proportion of SOC stock stored in a) particulate organic matter (POM), b) dissolved OC (DOC), c) residual SOC (rSOC), d) silt and clay (s+c), e) s+c less rSOC and f) sand and stable aggregate (S+A) isolated SOM fractions as a measure of fraction sensitivity to a land-use change from four different long-term tillage treatments to grassland at three depths (0 – 6 cm, 18 – 24 cm and 30 – 36 cm).

The POM fraction was consistently more sensitive than the total SOC to land-use change (the slope of the regression line is > 1) concurrent with large proportional changes in the C stored in this fraction (25–300%). The DOC fraction was also more sensitive than the total SOC for all treatments except DP which was as sensitive as the whole soil (slope of 1.1). The rSOC fraction was slightly more sensitive than the whole soil (slope range from 1.1–1.3) in all treatments except the DP treatment which was slightly less sensitive (slope of 0.9); all other fractions were less sensitive than the total SOC.

The rSOC fraction, which is thought to be a biologically recalcitrant pool (allocated to the inert pool in the RothC model) was, surprisingly, more sensitive than the s+c-rSOC fraction

and was also more sensitive than the total SOC for all treatments except for DP which was slightly less sensitive (slope 0.9). However, similar results were reported by Poeplau and Don (2013) for soils under a range of land use changes.

Table 5.7: Regression equations, R^2 values and significance level of the regression for the relative (%) change in equivalent soil mass soil organic C (SOC) stocks in chisel plough, direct drill, deep plough, shallow plough and all treatments against the relative change in the proportion of SOC stock stored in particulate organic matter (POM) chisel plough, dissolved organic C (DOC), residual SOC (rSOC), silt and clay (s+c), s+c less rSOC (s+c-rSOC) and sand and aggregate (S+A) SOM fractions as a measure of fraction sensitivity to land-use change. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns – not significant.

		Fraction					
		POM	DOC	rSOC	s+c	s+c-rSOC	S+A
All Treatments	Regression	$y = 3.37x$	$y = 2.02x$	$y = 1.18x$	$y = 0.64x$	$y = -0.46x$	$y = 0.28x$
	R^2	0.73	0.67	0.79	0.72	0.18	0.09
	Significance	***	***	***	***	*	ns
Chisel plough	Regression	$y = 5.31x$	$y = 2.32x$	$y = 1.10x$	$y = 0.48x$	$y = -0.39x$	$y = 0.50x$
	R^2	0.82	0.55	0.61	0.28	0.28	0.11
	Significance	***	*	*	ns	ns	ns
Direct drill	Regression	$y = 3.76$	$y = 2.78x$	$y = 1.34x$	$y = 0.63x$	$y = -0.37x$	$y = -0.62x$
	R^2	0.88	0.93	0.77	0.94	0.30	0.59
	Significance	**	**	*	**	ns	ns
Deep plough	Regression	$y = 1.96x$	$y = 1.06x$	$y = 0.94x$	$y = 0.76x$	$y = 0.34x$	$y = 0.54x$
	R^2	0.63	0.33	0.75	0.86	0.12	0.38
	Significance	*	ns	*	***	ns	ns
Shallow plough	Regression	$y = 2.16x$	$y = 1.83x$	$y = 1.11x$	$y = 0.72x$	$y = -1.33x$	$y = 0.40x$
	R^2	0.55	0.70	0.95	0.66	0.53	0.29
	Significance	*	**	***	**	*	ns

5.3.8 Summary of results

Some clear differences between SOC stocks and fraction sensitivities after land-use change from different tillage methods to grassland were detected. The most noteworthy results were that although there was an overall increase in ESM SOC stocks from 1975 to 2014 inversion tillage treatments (DP and CP) at some depths displayed losses whilst the corresponding depth in the non-inversion tillage treatment (CP and DD) had increases. This served to even out the SOC stocks across the field.

There was a general increase in the relative mass of the S+A fraction for 1975 to 2014, but this was not accompanied by a corresponding increase in the relative SOC content of this fraction. Although there was no increase in the relative SOC in the s+c fraction, there was a significant increase in C:N from 1975 to 2014, accompanied by a corresponding decrease in the C:N of the S+A fraction. This indicates that fresh OM was being preferentially stored in the s+c fraction.

Preliminary FT-IR analysis of POM fractions showed a decrease in the level of humification within this fraction.

As expected, the labile fractions were the most sensitive to land-use change. However, the rSOC fraction was surprisingly more sensitive than expected. There were also differences in the sensitivity of fractions even between different tillage treatments.

5.4 Discussion

5.4.1 SOC stocks and C distribution between fractions

Guo and Gifford (2002) carried out a meta-study of 76 publications which measured the change in SOC stocks after a land use change from crop to pasture. Across studies, they calculated an average increase in SOC stock of 19% and that the magnitude of the effect was sensitive to the depth to which soils were sampled (i.e. the effect decreased with depth). This is within the range of the increases measured for all tillage treatments in this study, however the more intensive (inversion) treatments had SOC stock changes of -9.6 to +7.5% (SP and DP, respectively). For these two treatments the SOC stocks decreased sharply between 24 and 36 cm from 1975 to 2014, -37 and -27% for SP and DP, respectively. The higher SOC stock in 1975 may have been caused by increased fresh C inputs of SOC at these depths during inversion tillage.

The most notable change in the distribution of SOC stock between fractions was a decrease in the proportion of total SOC contained within the S+A fraction from 1975 to 2014. Despite this, the relative mass of the S+A fraction increased. The mass increase is in line with the expectation that cessation of tillage allows the prolonged formation of macro-aggregates (Six *et al*, 2000; John *et al*, 2005; O'Brien and Jastrow, 2013), which in turn increases the formation of the more stable micro-aggregates where C is physico-chemically stabilised. This lag between the improvement in aggregation and the accumulation of C physically protected in aggregates has been observed previously (DeGryze *et al*, 2004, O'Brien and Jastrow, 2013).

Along with the increase in S+A mass, there was a significant decrease in the C:N from 1975 to 2014, and although there was no significant increase in the proportion of ESM SOC stock stored in the s+c fraction, there was a significant increase in the C:N, indicating more decomposed material. As these are the two proportionately largest fractions, this suggests that fresh OM was preferentially being physically protected in micro-aggregates whilst older material was being adsorbed on to minerals. Lehmann *et al* (2007) found evidence that occlusion within stable micro-aggregates was a secondary process which is initiated by adsorption of OM on to mineral surfaces.

It is unfortunate that archived samples were not available from the time that the tillage experiment was ceased, preventing C accumulation rates from being calculated. The tillage in 2013 and 2014 may have also caused some SOC losses which are impossible to quantify. Conant *et al* (2007) estimated that the loss of C from just one tillage event in no-tillage agricultural systems could lead to losses of between 1 and 11% of SOC stock, indicating that

the grassland in this study may have lost a significant amount of C due to these recent tillage events.

5.4.2 Sensitivity of SOM fractions

There have been few studies which have used isolated SOM fractions to assess the resilience of soil C to land use change. In this study, the labile POM and DOC fractions were found to be the most sensitive to a land-use change from chisel plough, direct drill, deep plough or shallow plough arable management to grassland. Poeplau and Don (2013) fractionated soil from 24 paired plots with various divergent land use changes (from cropland to grassland or woodland and grassland to cropland or forest) and found that the sensitivity of the fractions decreased in the order $POM > S+A > DOC \approx s+c-rSOC > rSOC$. Other studies have also found that POM was the most sensitive fraction to land use change (Conant *et al*, 2004, Franzluebbers *et al*, 2002; Plaza-Bonilla *et al*, 2014). However, this result has not been universal in the literature: Leifeld and Kögel-Knabner (2005) found that the stable aggregate fraction ($> 20 \mu m$ after sonication and density separation) was most sensitive whilst there was no difference in the proportion of total C stored within POM between plots where a land-use change from arable to grassland had occurred seven years previously, compared to arable, grassland or a grassland to arable land-use change (i.e. POM was not sensitive to land-use change). Don *et al* (2009) found no difference in POM C concentrations between paired cropland and grassland sites and Simonsson *et al* (2014) found that POM was not a good indicator of SOC stock changes over decadal time scales due to the spatial variability of POM in the field.

Sohi *et al* (2010) assessed the intra-aggregate light fraction (LF) (POM occluded in aggregates) as a sensitive indicator for SOC stock changes. They compared the difference in total SOC stock and intra-aggregate light fraction C in paired plots with divergent land management and found that the change in intra-aggregate LF C was proportional to that of the total SOC stock (17–30 years after management change). DeGryze *et al* (2004) also found the POM occluded in micro-aggregates was a sensitive indicator to land-use change. As POM is known to be spatially variable (Burke *et al*, 1999; Simonsson *et al*, 2014), perhaps extending the Zimmermann *et al* method to isolate the intra-aggregate and free light fractions separately would improve the assessment of fraction sensitivity.

A surprisingly high sensitivity (greater than total SOC) of the NaOCl resistant (rSOC) fraction, defined as the inert pool in the RothC model, was observed by Spohn and Giani (2011) in an arable/pasture/forest chronosequence. Poeplau and Don (2013) reported an increase in the size of the rSOC fraction after a land-use change from arable to grassland, and surprisingly high sensitivity of the rSOC fraction to land-use changes between forest, arable and grassland, although less sensitive than the total SOC. However,

Poeplau and Don (2013) did not separate out the different land-use changes in their regression analysis and this study showed that even conversion from different tillage methods to grassland can result in different degrees of fraction sensitivity. Helfrich *et al* (2007) found that the size of the NaOCl resistant fraction was dependent on the land-use and was not a suitable predictor of the inert C fraction. Poeplau and Don (2013) concluded that the rSOC pool may indeed be sensitive to land-use change, but highly stable under steady state conditions.

The S+A and s+c-rSOC fractions were generally the least sensitive to land-use change in this study. Poeplau and Don (2013) found that the S+A fraction was more sensitive than the total SOC, DOC, s+c-rSOC and rSOC (slope of the regression line was 1.4). Zimmermann *et al* (2007c) found that the relative amount of SOC stored in the S+A fraction varied considerably between different land-uses (from 15% in arable land to 55% in alpine grassland), suggesting that this fraction is sensitive to land-use change through improved aggregation (see discussion above).

The s+c-rSOC fraction is considered to be an active fraction (Leifeld and Kögel-Knabner, 2005; Zimmermann *et al*, 2007c). However, there was a negative correlation between total ESM SOC stock and s+c-rSOC stock in all cases except the DP treatment, but particularly the SP treatment which was had a significantly negative correlation and was more sensitive than the whole soil. The C:N was also observed to increase from 1975 to 2014, suggesting an accumulation of decomposed material. It could be tentatively suggested that SOC was being quickly sequestered in the rSOC fraction from the s+c-rSOC fraction as the C:N of this fraction was considerably higher (Table 5.6).

5.4.3 Limitations

A number of weaknesses have been identified in this study: 1. uncertainty in changes to SOC in archived samples during storage, 2. absence of samples from 1992, when the tillage experiment was ceased, 3. two tillage events prior to sampling in 2014, 4. the inability to statistically analyse all C:N ratios due to the very low N concentrations in some soil fractions and 5. lack of true replication due to the bulking of samples in 1975. However, it was possible to identify some clear differences which allowed an assessment of the changes in SOC stability and sensitivity after land use change to be made.

5.5 Conclusions

Overall, the soil examined in this study has shown some resilience in recovering from arable management practices. Mass equivalent SOC stocks had, on average, increased even though the inversion tillage treatments (DP and SP) had decreased C stocks below 24 cm and the overall effect was an evening out of SOC stocks across the field. The increased proportional

mass but decreased proportion of C in the S+A fraction shows that although soil structure was improved, increased physical protection of SOC lagged behind.

There are only a limited number of studies in the literature which address changes in SOC stability after land-use change. The results of such studies have not been universal although in general, as was found in this study, the labile fractions have been found to be most sensitive. The rSOC fraction has also often been found to be surprisingly more sensitive than the mineral bound s+c-rSOC and S+A fractions, questioning, as previous researchers have done (Rethemeyer *et al*, 2004; Poeplau and Don, 2013), the existence or the ability of fractionation methods to isolate an inert SOM fraction. More studies like the one presented here are required on a wide variety of soil textures and different land-use changes to establish general principles for estimating changes in SOC turnover after land-use change.

6 Soil fractions and respiration – direct assessment of physical protection and its influence on temperature sensitivity

6.1 Introduction

Global soil respiration has recently been estimated at 91 Pg C yr⁻¹ (Hashimoto *et al*, 2015), around ten times the C emissions from fossil fuel combustion (IPCC, 2014), with rates partially controlled by the stability of soil organic matter (SOM) in the soil. To be able to predict and project the impacts of land management practices such as tillage, as well as future climate change on soil respiration, it is important to establish the distribution of soil organic matter (SOM) reactivity in the soil matrix (von Lützow *et al*, 2008). Three mechanisms of SOM protection are commonly identified as factors controlling its stability: inherent chemical recalcitrance, physical protection in aggregates and adsorption to soil mineral surfaces (Six *et al*, 2000; Krull *et al*, 2003; von Lützow *et al*, 2006).

In recent years it has become increasingly clear that the traditional view of all stabilised SOM being chemically recalcitrant is incorrect (Mikutta *et al*, 2006; Marschner *et al*, 2008; Dungait *et al*, 2012; Kleber *et al*, 2011; Schmidt *et al*, 2011), and so the research focus has shifted to the role of physical protection of SOM in aggregates (von Lützow *et al*, 2006; Chevallier *et al*, 2010; Zimmermann *et al*, 2012; Dungait *et al*, 2012). Aggregation restricts access of microbes and microbial enzymes to the substrate and limits oxygen diffusion into the intra-aggregate space, reducing aerobic respiration (von Lützow *et al*, 2006); however, aggregates are susceptible to disturbance by tillage (see Section 4.1).

If physical protection alone is important it would be expected that physically protected SOM would have a lower turnover than unprotected SOM, and if the protection was removed (by aggregates being broken apart during land management practices such as tillage, for example) the availability and reactivity would increase. However, if physical location alone is important, SOM would have the same composition in all positions in the soil matrix but turn over at different rates. The use of soil fractionation methods to separate SOM into pools with different physical properties has shown that SOM with different chemical composition are found in different locations within the soil matrix (Sohi *et al*, 2001). This coupling of physical position in the soil matrix and the chemical properties of SOM has driven the debate over the relative importance of chemical and physical protection.

While soil fractionation methods have been used primarily to quantify and chemically characterise SOM fractions, less attention has been given to potential differences in the biological utilisation of isolated SOM fractions (Sollins *et al*, 1984; Whalen *et al*, 2000; Swanston *et al*, 2002; Burns, 2011). In principle, summing the respiration of isolated SOM

fractions and comparing to whole soil should give a net measurement of physical protection. However, care needs to be taken to account for methodological artefacts, particularly caused by ultrasonic dispersion and exposure to chemicals during the fractionation procedure (Elliott and Cambardella, 1991; Cambardella and Elliott, 1993; Amelung and Zech, 1999).

Global temperature increases have caused an estimated soil respiration response of $+0.1 \text{ Pg C yr}^{-1}$ (Bond-Lamberty and Thomson, 2010) increasing atmospheric CO_2 levels and initiating a positive feedback loop (Davidson and Janssens, 2006). Research into the temperature sensitivity of SOM has focused mainly on the chemical composition, or quality, of SOM. Enzyme kinetic theory predicts that the activation energy, and so the temperature sensitivity of SOM should increase with chemical recalcitrance of organic material (Bosatta and Ågren, 1999) and indeed some results have confirmed this (Feng and Simpson, 2008; Karhu *et al*, 2010; Wagai *et al*, 2013; Lefèvre *et al*, 2014). However, conflicting results have been reported in the literature which have shown no impact of temperature on SOM respiration rate (Giardina and Ryan, 2000), no difference in the response of labile and recalcitrant SOM to temperature (Fang *et al*, 2005; Conen *et al*, 2006) and a negative correlation between aromatic content and temperature sensitivity (Erghan *et al*, 2013).

It should be expected that if microbial access to SOM is restricted (i.e. by aggregation) then the removal of this restriction (by tillage, for example) would lead to increased temperature sensitivity as substrate concentration is no longer limiting (Davidson and Janssens, 2006). Gilllabel *et al* (2010) found evidence that physical protection of SOM reduced its temperature sensitivity during a long term incubation of topsoil and subsoil. The subsoil SOM was deemed to have lower lability and greater physical protection than the topsoil SOM and had a much lower response to temperature than the topsoil (Q_{10} values of ~ 1 and $1.9\text{--}3.8$, respectively).

Zimmermann *et al* (2012) found that the relative physical distribution of soil organic C (SOC) in the soil matrix correlated well with temperature sensitivity, but not to the absolute concentration of C in any fraction or to its chemical composition. This would suggest that the chemical or physical protection of SOM is more important than SOM quality in determining temperature sensitivity.

The main objective of this study was to assess the level of physical protection afforded to SOC by occlusion in aggregates by measuring its change in susceptibility to loss upon disturbance to aggregation caused by land management practices such as tillage. The experiment also aimed to determine how loss of physical protection affects the response of SOM respiration to increases in temperature. Respiration rate under controlled conditions is used as a proxy for SOC stability. We hypothesised that removing the physical protection would lead to increased respiration from intra-aggregate fractions and also to greater

temperature sensitivity. Isolated soil fractions were incubated separately, recombined and mixed in to whole soil at three different temperatures. Fractions were also recombined to form “reconstituted” soils, in part to address the possibility of artefacts from the fractionation procedure. If physical protection is re-established rapidly and there are no artefacts, then respiration should be equal to that of the whole soil.

6.2 Materials and Methods

6.2.1 Site Description

The soil chosen for this study was a moderately well drained sandy loam grassland from the Crichton Dairy Research Farm in Dumfries, UK (55°02.5'N, 3°35.3'W). The site was permanent cut grassland that had not received lime for over 20 years and had no long-term history of organic manure application (Bell *et al*, 2015a). Soils belonged to the Holywood association and Crichton Midpark soil series (classified as Eutric Cambisols according to the FAO world reference base for soils (FAO, 1990)) which are non-calcareous and derived from red sandstone parent material. Soil from this site was also used in Chapter 4 where further chemical and climatic information for the site can be seen (Section 4.2.1). Perennial grassland was chosen for this direct examination of physical protection of SOM because the physical structure of grassland soil is not disrupted by tillage, and so the effect of removing physical protection mechanisms should be greater.

6.2.2 Preparation of whole soil and fractions

Soil was collected in August 2014 to a depth of 10 cm, sieved to < 4 mm, air dried and stored at room temperature in sealed plastic bags and fractionations were carried out within one month. The physical fractionation method of Sohi *et al* (2001) (methodology is described in Section 3.2.1) was chosen for this study as the three SOM fractions recovered using this method had previously shown distinctly different radiocarbon ages for this soil (see Section 4.3).

Soil fractionations were continued until sufficient amounts of all fractions for each of the separate fractions, recombined fractions and fractions mixed into unfractionated whole soil treatments had been gathered (treatments and fraction masses are shown in Table 6.1). All fractions were bulked, placed on a filter paper, and rinsed firstly with a 0.01 M CaCl₂ solution (~200 ml for each batch of free and intra-aggregate LF, and ~500 ml for the organo-mineral fraction) to release Na⁺ ions, and then continuously with ~3 l of deionised water for free and intra-aggregate LFs and ~5 l for organo-mineral fractions. Fractions were then dried in a 40 °C oven.

A soil inoculum was added to all treatments to ensure a similar microbial community in each sample at the beginning of incubation and to preclude imbalance of microbial activity

between samples arising from micronutrient deficiencies (Cheng *et al.*, 2008). The inoculum was prepared as follows: 1 l of deionised water was added to 100 g of fresh whole soil collected from the same site. The suspension was placed on an orbital shaker (200 rpm) for 1 hour along with four ball bearings, to break apart aggregates and liberate occluded microbial colonies. Microbes were then concentrated by centrifuging at 2200 g for 15 minutes after which the solution was filtered at 5 μm . Micronutrients were added to the inoculum following the formulation of Cheng *et al.* (2008): 4 mM NH_3NO_3 , 4 mM CaCl_2 , 2 mM KH_2PO_4 , 1 mM K_2SO_4 , 1 mM MgSO_4 , 25 mM MnSO_4 , 2 mM ZnSO_4 , 0.5 mM CuSO_4 and 0.5 mM Na_2MoO_4 . The resulting solution had a dissolved organic C content of 27.8 $\mu\text{g C l}^{-1}$ and was used to adjust the whole soil, organo-mineral fraction and sand to 60% water filled pore space. The free LF and intra-aggregate LF, whose masses made only a minor contribution to whole soil mass (see Table 6.1) were assumed to have negligible water holding capacity and so were not adjusted for moisture.

6.2.3 Chemical characterisation of whole soil and fractions

Whole soil, free LF, intra-aggregate LF, organo-mineral fraction and sand samples were combusted and analysed for organic C (OC) and total nitrogen (TN) using a Flash 2000 (Thermo Scientific) elemental analyser.

Chemical characterisation using FT-IR spectroscopy was carried out on isolated fractions from soil collected from the same site (see Section 4.2.3 for details). However, this soil was collected in June 2012 and so some slight differences might be expected.

6.2.4 Incubation of whole soil and fractions

There were 12 treatments consisting of different permutations of soil, sand and isolated fractions (Table 6.1), and one sand control, all of which had a mass of 5 g in total, were placed in 22 ml glass vials. The small vial volume and masses of treatments were chosen as the two light fractions make up only a very small proportion of the whole soil and isolating them is time and resource intensive. Therefore, a mass of 5 g was chosen as a compromise between the effort required to isolate a sufficient amount of fraction for each treatment whilst maintaining a sufficient volume of treatment to produce measurable CO_2 fluxes. Additionally, the small vials allowed CO_2 fluxes to be measured directly from the vials by placing them on the GC (see below). Inoculum infused, acid-washed quartz sand was incubated as a control. Combinations of unprotected fractions were made according to their average mass ratios in the whole soil (following Swanston *et al.*, 2002); these were 98.1, 1.6 and 0.4% by mass for the organo-mineral, free LF and intra-aggregate LF, respectively. Isolated fractions were also mixed into whole soil according to their mass ratios as found in the whole soil. This resulted in twice the normal mass of each fraction being present – half in its normal position within the soil matrix, and half isolated. Acid washed and autoclaved

carbonate free white quartz sand (0.02 – 2 mm) was used as a substitute for fractions where appropriate. Whole soil soaked in NaI was included as a control to assess the inhibitory effect of NaI on microbial respiration.

There were 15 blocks of treatments, each containing one treatment replicate, but these were not all placed in the incubator simultaneously (Figure 6.1). All incubations began with a two week pre-incubation period at 20 °C. Firstly, nine blocks (hereby referred to as s15 blocks) were placed into an incubator at 15 °C for three weeks. The incubation temperature then increased to 25 and 35 °C, and blocks were incubated for three weeks at each temperature. At each temperature change three blocks were removed from the incubator and replaced with three fresh blocks (s25 and s35 blocks at 25 °C and 35 °C, respectively). The s25 and s35 blocks were pre-incubated in the same manner as the s15 blocks and were used to assess differences in the respiration caused by the sequential incubation method.

Table 6.1: Target masses for soil/sand/fraction to four decimal places. Isolated fractions were added to whole soils in the proportions by mass in which they are found in the soil. Reconstituted soils have all combinations of isolated fractions in the proportions found in the whole soil by mass. LF - light fraction, o-min - organo-mineral fraction.

Sample	Mass soil/sand/fraction (g)						Inoculum C (mg C)
	Whole soil	Sand	Free LF	Intra- aggregat e LF	O-min	Total	
Whole soil + sand	2.6214	2.3786	-	-	-	5	0.08
Whole soil	5	-	-	-	-	5	0.12
Whole soil + free LF	4.9726	-	0.0274	-	-	5	0.12
Whole soil + intra- aggregate LF	4.9814	-	-	0.0186	-	5	0.12
Whole soil + o-min	2.6214	-	-	-	2.3786	5	0.11
All fractions	-	-	0.0301	0.0204	4.9495	5	0.08
Free LF + sand	-	4.9699	0.0301	-	-	5	0.05
Intra-aggregate LF + sand	-	4.9796	-	0.0204	-	5	0.05
o-min + sand	-	0.0505	-	-	4.9495	5	0.11
Free LF + intra-aggregate LF + sand	-	4.9495	0.0301	0.0204	-	5	0.05
Free LF + o-min + sand	-	0.0203	0.0301	-	4.9495	5	0.11
Intra-aggregate LF + o-min + sand	-	0.0301	-	0.0204	4.9495	5	0.11

Vials were covered with parafilm throughout the incubation period to prevent moisture loss but allow free gas exchange. An open jar of water was also kept in the incubators to maintain humidity. During test incubations the maximum moisture loss was from a whole soil sample at 35 °C where 0.21 ml (equivalent to 5% of added moisture) was evaporated over a three

week period. Vials were closed for 24 hours and placed directly onto a gas chromatograph (GC) for determination of CO₂ flux by headspace gas sampling twice per week. During these closure periods parafilm was replaced with gas tight lids fitted with rubber septa.

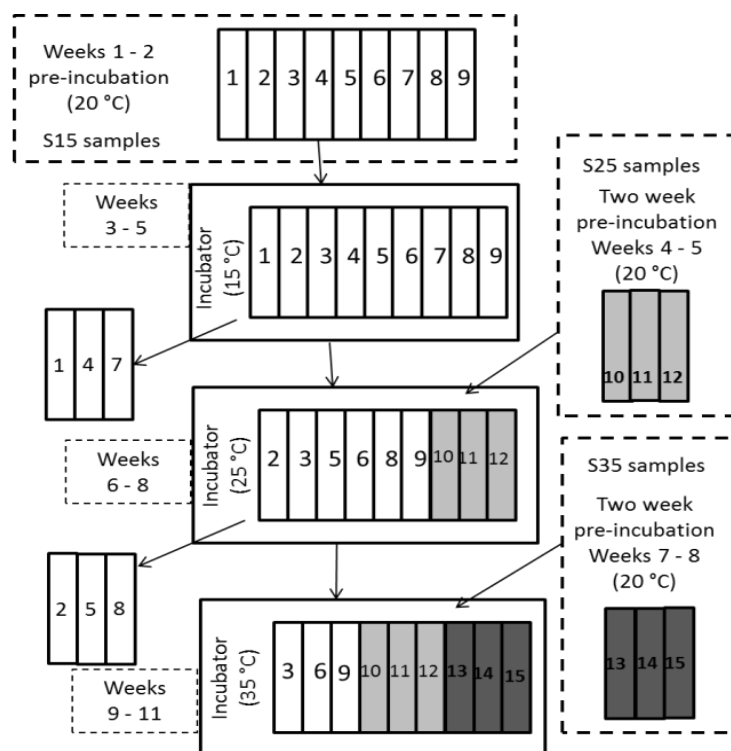


Figure 6.1: Experimental design for the respiration and temperature sensitivity incubations. Numbered rectangles represent blocks with one replicate of each treatment (see Table 6.1).

6.2.5 Analysis of gas samples

Gas samples were analysed for N₂O, CO₂ and CH₄ using an Agilent 7890A Gas Chromatograph (GC) fitted with electron capture, flame ionisation and thermal conductivity detectors (Agilent Technologies, Berkshire, UK) and a CTC Analytics COMBI PAL autosampler (CTC Analytics, Hampshire, UK). The GC gas peak area responses were calibrated (calibration curves were linear for CO₂ and CH₄, quadratic for N₂O) using four certified standard gas mixtures (BOC Industrial Gases, UK) (see Table 6.2).

Table 6.2: Certified standard gas mixture concentrations used to calibrate the gas chromatograph.

Certified standard	N ₂ O (ppm)	CO ₂ (ppm)	CH ₄ (ppm)
1	0.35	390	2
2	1.1	1093	9.7
3	5.1	5262	5.2
4	10.7	10100	22.1

6.2.6 Calculations

Inoculum C added to the whole soil, the organo-mineral fraction and sand was not included in the calculation. To account for any respiration attributable to inoculum C, an amount of respiration was subtracted which was relative to that of the sand only control. All respiration from the sand control was assumed to be attributable to the microbial utilisation of inoculum C.

Temperature sensitivity was quantified by calculating Q_{10} values simply as a function of the respiration rates at 15, 25 and 35 °C using the following equation (Leifeld and Fuhrer, 2005):

$$Q_{10} = (R_2 / R_1)^{(10 / (T_2 - T_1))}$$

Where R_1 and R_2 are the respiration rates at the lower and higher temperature, respectively, and T_1 and T_2 are the lower and higher temperatures in °C. Q_{10} values were calculated between 15 and 25 °C, and 25 and 35 °C and also from the sequentially incubated samples as well as from the sequential and ‘fresh’ blocks (to allow an inference to be made about the influence of the quality of the material remaining in the sequentially incubated replicates).

6.2.7 Statistical Analyses

All statistics were carried out using ANOVA followed by a multiple comparisons post hoc test in Genstat (15th edition). Significance was determined at $p < 0.05$.

6.3 Results

6.3.1 C and N analysis of SOM fractions

Contents of C and N followed the order free LF > intra-aggregate LF > whole soil > organo-mineral fraction. Calculated C:N for the isolated fractions were slightly lower (not significantly) than those measured for the soil collected from the same site in 2012 (see Section 4.2.1). The intra-aggregate LF had a significantly higher C:N than the whole soil and

organo-mineral fraction, but not the free LF. Any N present in the quartz acid-washed sand were below the detection limit of the instrument (Table 6.3).

Table 6.3: Concentrations of organic C and total N (g kg^{-1}) and calculated C:N ratios for the whole soil, isolated soil fractions and sand. Standard errors are shown in parenthesis where ≥ 0.01 . LF - light fraction, LOD - limit of detection. Values in the C:N ratio column followed by a common letter were not significantly different from each other (ANOVA, $p < 0.05$).

	Concentration (g kg^{-1})		
	Organic C	Total N	Average C:N
Whole soil	2.65 (0.09)	31.5 (1.5)	11.9 (0.2)a
Organo-mineral	1.89 (0.08)	21.4 (0.9)	11.3a
free LF	15.5 (0.2)	276 (28)	17.8 (1.5)ab
intra-aggregate LF	7.6 (0.3)	158 (21)	20.6 (2.0)b
sand	0.01	< LOD	-

Higher soil C:N indicate that soils are less decomposed and soil C:N usually decreases with soil depth (John *et al.*, 2005). The high C:N for the intra-aggregate LF suggests that the SOM in the intra-aggregate LF (C:N ~21) is slightly less decomposed, and therefore more labile than the free LF (C:N ~18). Indeed results from the incubations indicated that the intra-aggregate LF had significantly higher CO_2 emissions in all instances, except at 35 °C for the s15 and s35 samples where respiration from the free LF was not significantly lower than the intra-aggregate LF. This supports the idea that substrate decomposition is controlled by the accessibility of C to microbes as well as inherent chemical recalcitrance.

Results from the FT-IR spectroscopy are presented in Section 4.3.4.

6.3.2 Cumulative CO_2 -C emissions from incubations

At all temperatures, respiration rates were generally significantly lower (ranges in $\text{mg CO}_2\text{-C g}^{-1} \text{C}_i \text{ d}^{-1}$ for s15 and s25 sample sets: 0.00 – 0.02, and for s35 sample sets: 0.01–0.04) at the end of the 3 week incubation than at the beginning (ranges in $\text{mg CO}_2\text{-C g}^{-1} \text{C}_i \text{ d}^{-1}$ for the s15, s25 and s35 sample sets: 0.01–0.05, 0.01–0.08 and 0.03–0.12, respectively) ($p < 0.01$, repeated measures ANOVA) (see Appendix 4). The only exception was for the s15 samples at 15 °C, where the respiration rate was similar at the beginning and end of the 3 week period. There was generally a significant interaction between treatment and incubation time ($p < 0.05$), suggesting that the rates of respiration did not follow the same pattern in different treatments, consistent with results found by Swanston *et al* (2002).

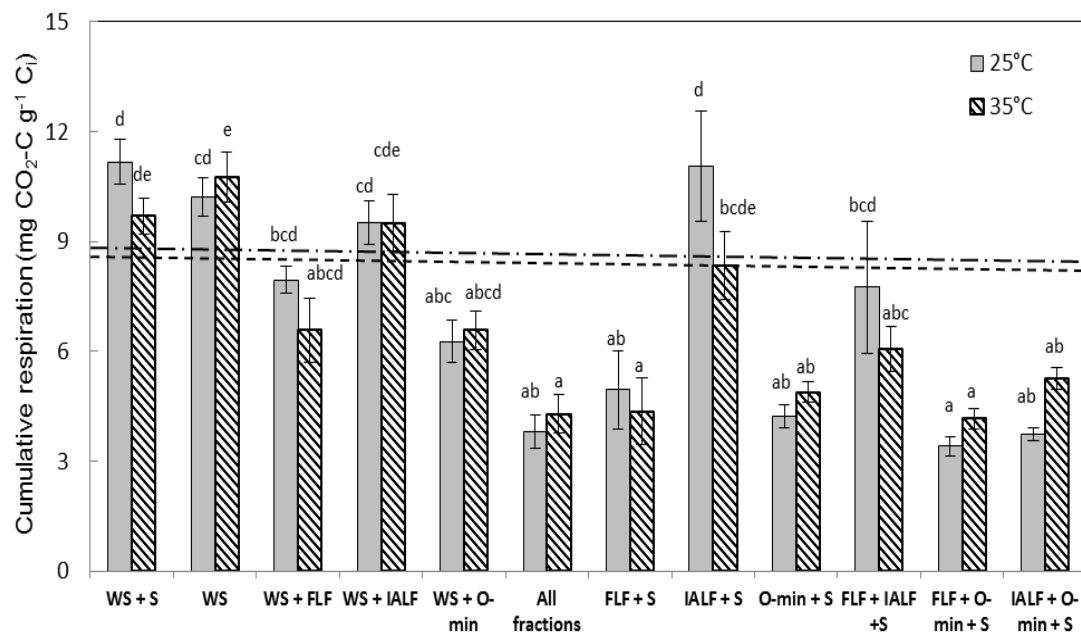
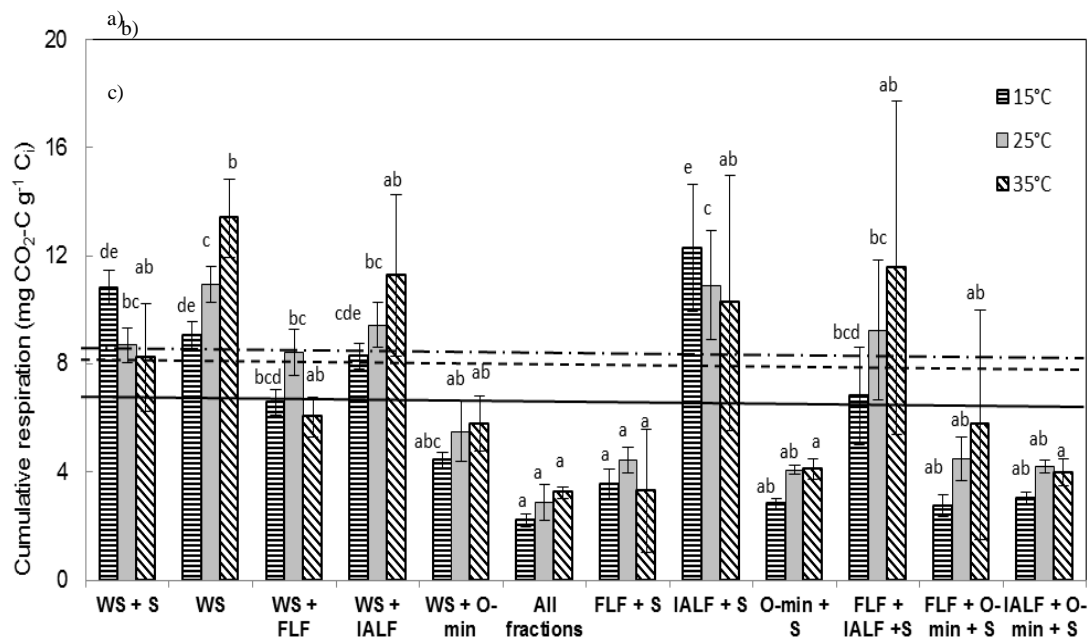
Cumulative emissions from the individual isolated fraction + sand and whole soil + fraction treatments generally decreased in the order intra-aggregate LF > free LF > organo-mineral for all sample sets at all temperatures (Figure 6.2). The intra-aggregate LF + sand treatment had cumulative emissions which were consistently as high as whole soil at all temperature in all sample sets. The whole soil treatment consistently had significantly higher cumulative emissions than the all fractions treatment.

Respiration from the sand only control over the full incubation periods of the s15, s25 and s35 sample sets was $0.08 \pm 0.01\%$ (~0.05 mg C, over nine weeks), 0.05% (~ 0.02 mg C, over six weeks) and 0.02% (~0.01 mg C, over three weeks), respectively, of the inoculum C added (~53 mg).

For the s15 sample sets, the CO₂-C emissions from the intra-aggregate LF + sand treatment were significantly higher than all other isolated fraction + sand treatments at 15 °C, and at 25 °C except for the free LF + intra-aggregate LF + sand treatment (Figure 6.2). Respiration from the whole soil + isolated fraction treatments were suppressed relative to the whole soil, but only significantly for the whole soil + organo-mineral treatment at 15 °C.

The pattern of CO₂-C emissions from the s25 sample sets (Figure 6.2) and the s35 sample sets (Figure 6.2) were similar to the s15 sample sets (Figure 6.2). Notable differences in emission trends between the s35 sample set and the s15 and s25 sample sets were that the whole soil + intra-aggregate LF treatment had the highest CO₂-C emissions, the free LF + intra-aggregate LF + sand treatment emissions were low and the free LF + sand treatment emissions were high compared with trends found for the corresponding s15 and s25 sample sets (Figure 6.2).

Within the s15, but not the s25, sample sets there was a significant difference between cumulative CO₂-C emissions within treatments at different temperatures (repeated measures ANOVA, $p < 0.05$), but there was no interaction between temperature and treatment.



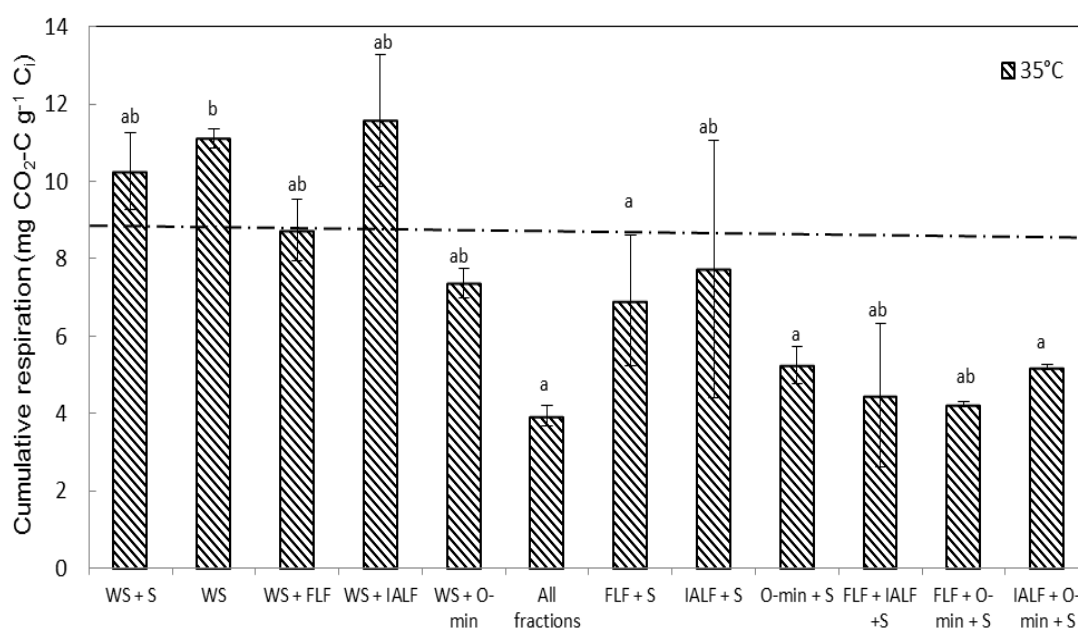


Figure 6.2: Cumulative CO₂-C emissions per gram of initial C (C_i) for control, whole soil + fraction treatments and reconstituted soil combinations from a) s15 samples sequentially incubated from 15 °C (21 days at each of 15 (n=9), 25 (n=6) and 35 °C (n=3)), b) s25 samples sequentially incubated from 25 °C (21 days at each of 25 and 35 °C (n=3)) and c) s35 samples sequentially incubated at 35 °C for 21 days (n=3). Treatments at the same temperature which have no common letters above were significantly different from each other (ANOVA, $p < 0.05$). The solid, dashed and dot-dashed lines represent the cumulative CO₂-C emissions at 15, 25 and 35 °C, respectively, from whole soil soaked in NaI and then rinsed thoroughly to demonstrate the impact of NaI on microbial respiration. WS – whole soil, S – sand, FLF – free light fraction, IALF – intra-aggregate light fraction and o-min - organo-mineral.

6.3.3 Summing cumulative CO₂-C emissions from isolated fractions

Summing average emissions from individual isolated fraction + sand treatments gave consistently higher emissions than their equivalent fraction combination treatments for all sample sets at all temperatures (Table 6.4). The sum of the three individual fraction + sand treatments had the greatest differences being 4.1–8.4 times greater than the all fractions treatment. However, the all fraction treatment emitted only 0.2–0.3 times as much as the whole soil at any temperature. Generally the difference between summed fractions and their equivalent treatments reduced after temperature increases within samples sets.

Table 6.4: Relative magnitude of average cumulative CO₂-C emissions of summed individual isolated fraction + sand treatments and equivalent recombined isolated fraction combination treatments, and compared to whole soil for the reconstituted soil treatment and for all three summed individual isolated fraction + sand treatments. s15 samples were sequentially incubated from 15 °C to 25 and 35 °C, s25 samples were sequentially incubated from 25 °C and 35 °C and s35 samples were incubated only at 35 °C (3 weeks at each temperature for all samples). LF – light fraction, S – sand, o-min – organo-mineral.

Treatment	s15 samples (°C)			s25 samples (°C)		s35 samples (°C)
	15	25	35	25	35	35
Free LF + S and intra-aggregate LF + S	2.3	1.7	1.2	2.1	2.1	3.3
Free LF + S and o-min + S	2.3	1.9	1.3	2.7	2.2	2.9
Intra-aggregate LF + S and o-min + S	5.0	3.6	3.6	4.1	2.5	2.5
Free LF + S, intra-aggregate LF + S and o-min + S	8.4	6.8	5.5	5.3	4.1	5.1
Reconstituted soil vs whole soil	2.1	1.8	1.3	2.0	1.6	1.8
Free LF + S, intra-aggregate LF + S and o-min + S vs whole soil	0.2	0.3	0.2	0.4	0.4	0.4

6.3.4 Considering the proportion of initial C respired

When expressing CO₂-C emissions from all treatments in terms of the proportion of C_i respired, the differences between treatments were of course consistent with those observed when considering the absolute emissions per g C (Table 6.5).

The whole soil had the greatest total % C_i respired for the s15 and s25 sample sets (~3 and ~2% C_i, respectively) and the whole soil + intra-aggregate LF treatment had slightly higher % C_i respired than the whole soil for the s35 sample set. The intra-aggregate LF + sand and free LF + intra-aggregate LF + sand treatments had total % C_i respiration which were as high as the whole soil in the s15 sample set, and the total % C_i respiration for the intra-aggregate LF + sand treatment was also as high as the whole soil in the s25 sample set.

Table 6.5: Average C mineralisation at each temperature and average total C mineralisation expressed as a percentage of the initial C (C_i) from samples sequentially incubated at 15, 25 and 35 °C (s15 samples), samples sequentially incubated at 25 and 35 °C (s25 samples) and samples incubated only at 35 °C (s35 samples). Samples were incubated at each relevant temperature for 21 days. Standard errors are shown in parenthesis where the error was > 0.00%. Values in the same column followed by common letters were not significantly different from each other (p < 0.05). LF = light fraction.

Treatment	s15 samples				s25 samples			s35 samples
	% C _i mineralised at 15 C	% C _i mineralised at 25 C	% C _i mineralised at 35 C	Total % C _i mineralised	% C _i mineralised at 25 C	% C _i mineralised at 35 C	Total % C _i mineralised	Total % C _i mineralised
Whole soil + sand	1.08 (0.06)de	0.87 (0.07)cd	0.83 (0.08)abc	2.84 (0.26)cde	1.12 (0.06)e	0.97 (0.05)cd	2.09 (0.11)e	1.03 (0.10)
Whole soil only	0.90 (0.05)de	1.09 (0.07)d	1.34 (0.03)c	3.26 (0.05)e	1.02 (0.05)e	1.08 (0.07)d	2.10 (0.12)e	1.11 (0.03)
Whole soil + free LF	0.66 (0.05)bcd	0.84 (0.09)bcd	0.60 (0.03)abc	2.02 (0.04)bcde	0.80 (0.04)cde	0.66 (0.09)abc	1.45 (0.12)bcd	0.87 (0.08)
Whole soil + intra-aggregate LF	0.83 (0.05)cde	0.94 (0.08)cd	1.13 (0.17)bc	3.02 (0.24)de	0.95 (0.06)de	0.95 (0.08)cd	1.90 (0.14)de	1.16 (0.17)
Whole soil + organo-mineral	0.44 (0.03)abc	0.55 (0.11)abcd	0.58 (0.06)abc	1.70 (0.06)abcde	0.63 (0.06)bcd	0.66 (0.05)abc	1.29 (0.07)abc	0.74 (0.04)
All fractions	0.22 (0.02)a	0.29 (0.07)ab	0.32 (0.02)ab	0.93 (0.01)ab	0.38 (0.05)ab	0.43 (0.05)a	0.81 (0.10)a	0.39 (0.030)
free LF + sand	0.35 (0.06)ab	0.44 (0.05)abc	0.33 (0.13)ab	1.11 (0.27)abc	0.50 (0.11)bc	0.44 (0.09)a	0.93 (0.18)ab	0.69 (0.17)
intra-aggregate LF + sand	1.23 (0.25)e	1.09 (0.22)d	1.03 (0.24)bc	2.99 (0.80)e	0.96 (0.07)de	0.83 (0.08)bcd	1.80 (0.15)cde	0.77 (0.33)
Organo-mineral + sand	0.28 (0.02)ab	0.41 (0.02)abc	0.41 (0.03)ab	1.12 (0.04)ab	0.42 (0.03)bc	0.49 (0.03)ab	0.91 (0.03)ab	0.52 (0.05)
free LF + intra-aggregate LF + sand	0.68 (0.19)bcd	1.02 (0.29)cd	1.15 (0.34)bc	3.00 (0.58)de	0.78 (0.18)cde	0.61 (0.06)ab	1.38 (0.13)bcd	0.45 (0.18)
free LF + organo-mineral + sand	0.28 (0.04)ab	0.45 (0.08)abc	0.57 (0.26)abc	1.33 (0.40)abcd	0.34 (0.03)ab	0.42 (0.03)a	0.76 (0.05)a	0.42 (0.01)
intra-aggregate LF + organo-mineral + sand	0.30 (0.02)ab	0.42 (0.02)abc	0.40 (0.03)ab	1.10 (0.06)ab	0.37 (0.02)ab	0.53 (0.03)ab	0.90 (0.04)ab	0.52 (0.01)

6.3.5 Influence of NaI on respiration of whole soil and soil fractions

Two potential issues were identified during the analysis of the respiration and chemical characterisation data. Firstly, the sand was found to have a pH of 4.7 indicating that it had not been sufficiently rinsed after acid washing. Sleutal *et al* (2012) found that acidification of soil to pH 4.3 reduced C mineralisation and promoted fungal growth. This may have affected fluxes from samples containing sand. Secondly, the NaI saturated whole soil treatment cumulative emissions were suppressed by 82–99% compared to the whole soil treatment.

To address these issues, further incubations were conducted with the following three treatments: sand which had been rinsed continuously with deionised or distilled water until its pH returned to 7, whole soil and NaI saturated whole soil which was continuously rinsed with CaCl_2 and deionised water until the water ran completely clear (as performed for the isolated fractions). Treatments were inoculated as described in Section 6.2.2 and incubations were run in parallel at 15, 25 and 35 °C for twenty days; vials were capped continuously and vented by a hollow needle inserted through a rubber septum in the cap. Needles were removed during sampling periods and headspace CO_2 gas samples were measured by placing vials directly onto the GC as previously described. Cumulative CO_2 -C emissions are shown in Figure 6.3.

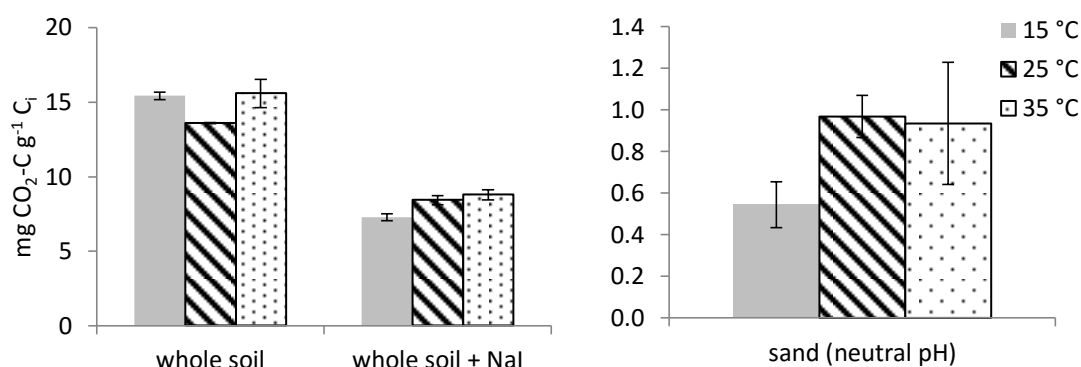


Figure 6.3: Cumulative CO_2 -C fluxes per gram of initial C (C_i) for whole soil, whole soil which had been saturated in NaI and then thoroughly rinsed and sand with pH 7.

The cumulative respiration from the thoroughly rinsed whole soil + NaI treatment was suppressed by 38 – 53% relative to the whole soil. This shows that even after thorough rinsing there is still a residual effect of the NaI on respiration. It is possible that residual NaI in the organo-mineral fraction might be responsible for suppressing emissions in the free LF + organo-min, intra-aggregate LF + organo-min and all fractions treatments, however, this does not seem likely for the free LF + sand, intra-aggregate LF + sand and free LF + intra-aggregate + LF + sand treatments.

The cumulative respiration rates from the neutral sand treatments were higher than from the acidic pH sand in the main experiment. Cumulative fluxes were 1.7, 2.8 and 5.9 times greater at 15, 25 and 35 °C, respectively indicating that respiration may have been suppressed in treatments containing sand. The sand CO₂-C emissions corresponded to ~0.1, ~0.1 and ~0.9% C_i mineralised (9 mg C added and 0.5 ± 0.1, 1 ± 0.1 and 0.9 ± 0.3 mg C mineralised at 15, 25 and 35 °C, respectively).

6.3.5 Temperature sensitivity of isolated soil fractions and reconstituted soil respiration

The median Q₁₀ ranged from 0.8–1.9 for all permutations of fraction, sand and soil for any method of calculation. Inferred Q₁₀ were highest when based on data collected immediately after each temperature increase and was significantly reduced ($p < 0.01$) with time for the temperature change from 25 to 35 °C in the s15 and s25 blocks, but not for the temperature change from 15 to 25 °C (Table 6.6). The Q₁₀ for s25 blocks immediately after the increase from 25 to 35 °C (day 4) were quite extreme, ranging from 57 for the whole soil + sand permutation to 111 for the intra-aggregate + organo-mineral + sand permutation. These extreme values skew the means for these samples (means for s15 sample Q₁₀ values are close to the median) and so only median values will be considered here. However, no significant differences in Q₁₀ persisted more than 14 days after a temperature change, either for amendment of sand or soil.

The average Q₁₀ values were surprisingly flat regardless of treatment (Table 6.6), with minor differences between permutations. Although the Q₁₀ was very high after a temperature change for the s15 recombined free LF + intra-aggregate LF + sand and the s25 intra-aggregate LF + sand, these values dropped rapidly and were converging with the other permutations by the end of each of the three week periods (lower value of Q₁₀ ranges).

Calculated Q₁₀ values were significantly different between s15 and s25 blocks for each measurement after the 25 to 35 °C temperature change. The level of significance decreased with time ($p < 0.001$ at day four, $p < 0.01$ at day 21).

Table 6.6: Range, mean and median Q_{10} values of samples sequentially incubated from 15 °C (s15 sample set, 15 to 25 °C, and 25 to 35 °C) and for samples sequentially incubated from 25 °C (s25 sample set, 25 to 35 °C), 21 days at each temperature. LF – light fraction, o-min – organo-mineral fraction. Median Q_{10} values with no common letters in a column are significantly different (one-way ANOVA, $p < 0.05$).

Treatment	s15 Q_{10} (15 - 25 °C)			s15 Q_{10} (25 - 35 °C)			s25 Q_{10} (25 - 35 °C)		
	Mean	Range	Median	Mean	Range	Median	Mean	Range	Median
Whole soil + sand	0.8	0.7 - 1.0	0.8a	1.0	0.9 - 1.2	0.9ab	10.4	0.9 - 57.3	0.9a
Whole soil	1.2	1.1 - 1.6	1.2ab	1.6	1.3 - 2.1	1.5b	12.7	1.1 - 69.6	1.2a
Whole soil + free LF	1.2	1.0 - 1.5	1.1a	0.9	0.8 - 1.1	0.9ab	12.1	0.8 - 67.3	0.9a
Whole soil + intra-aggregate LF	1.1	1.0 - 1.4	1.1a	1.2	1.1 - 1.5	1.2ab	11.7	1.0 - 64.4	1.0a
Whole soil + o-min	1.1	0.9 - 1.2	1.1ab	1.0	0.9 - 1.4	1.0ab	18.1	1.1 - 101	1.4a
free LF + intra-aggregate LF + o-min	1.1	0.9 - 1.5	1.0a	1.0	1.0 - 1.3	1.0ab	16.7	1.1 - 93.4	1.2a
free LF + sand	1.4	1.2 - 1.9	1.3ab	0.7	0.7 - 0.8	0.7a	11.1	0.9 - 60.5	1.2a
intra-aggregate LF + sand	1.4	1.2 - 1.8	1.3ab	0.8	0.7 - 0.8	0.8ab	14.5	1.6 - 77.8	1.9a
o-min + sand	1.2	1.0 - 1.4	1.2ab	1.3	1.0 - 1.8	1.2ab	19.8	1.2 - 110	1.4a
free LF + intra-aggregate LF + sand	2.0	1.5 - 3.2	1.9b	0.9	0.8 - 1.0	0.9ab	15.6	0.9 - 87.1	1.2a
free LF + o-min + sand	1.3	1.1 - 1.6	1.2ab	1.3	1.1 - 1.8	1.2ab	17.9	1.2 - 99.8	1.4a
intra-aggregate LF + o-min + sand	1.3	1.0 - 1.5	1.2ab	1.2	1.0 - 1.6	1.1ab	20.2	1.4 - 111	1.8a

It is also worthwhile considering Q_{10} values calculated using only the cumulative emissions from the initial incubation temperature from each set of samples, although these were not truly parallel as they were placed into the same incubator at temperature increases rather than being incubated at the same time in separate incubators. Q_{10} values were consistently lower when calculated in this way ($p < 0.001$ for the temperature change from 15 to 25 °C and $p < 0.01$ for the temperature increase from 25 to 35 °C).

6.4 Discussion

6.4.1 CO₂-C emissions from incubations

Previous studies have shown that removing the physical protection of SOC leads to an increased respiration rate (Balesdent *et al*, 2000; Six *et al*, 2002) but there have been no direct measurements of the effects of physical protection on C stability in using modellable SOM fractions.

The C respiration rates of the isolated intra-aggregate LF and the intra-aggregate LF + free LF treatments were generally consistently as high as the whole soil. This supports the theory that there is a labile component of soil which is protected from decomposition by physical protection within aggregates. Several studies have looked at how SOC is protected in aggregates by comparing intact aggregates with aggregates which have been crushed or ground to release occluded OM. Generally these studies have shown significant increases in respiration when aggregates were sufficiently disrupted compared to intact aggregates (Six *et al*, 2002; Pulleman and Marinissen, 2004; Chevallier *et al*, 2004; Plante *et al*, 2009; Goebel *et al*, 2009).

Therefore, the lack of any priming effect with the addition of free or intra-aggregate LF material to the whole soil, and indeed the suppression of emissions relative to the whole soil, was surprising. A similar response was observed by Whalen *et al* (2000) on addition of light fraction material ($< 1.6 \text{ g cm}^{-3}$, free and intra-aggregate LF) from 3 different soils, each under two types of land management, into their respective whole soils. In five of the six cases there was a small (not significant) suppression of C emissions compared to the whole soil treatments.

These ‘negative priming’ effects may be explained by shifts in the microbial community structure or function. The higher C:N ratio of the free and intra-aggregate LFs suggest that they are more labile than the whole soil and might be expected to be preferentially decomposed, and a greater proportion of C can be assimilated into the microbial biomass, leading to less of the substrate C being respired as CO₂ (Manzoni *et al*, 2012). However, it is possible that these fractions became quickly stabilised in the whole soil matrix. The whole soil + organo-mineral fraction treatment showed the largest and most persistent suppression of CO₂-C emissions relative to the whole soil, this is most likely due to the increased proportion of mineral bound C.

It has been suggested that residual NaI in isolated SOC fractions may be responsible for suppressed emissions: Na toxicity could have been restricting C mineralisation (Magid *et al*, 1996; Swanston *et al*, 2002; Crow *et al*, 2007; Burns, 2011). In this study summing any of the individually incubated isolated fractions gave a cumulative respiration which was consistently higher than any of the recombined equivalent treatments. This was due in large part to the intra-aggregate LF, which had cumulative respiration which was not significantly different from the whole soil for any set of samples at any temperature. However, the NaI saturated and NaI saturated and rinsed whole soil (investigated in the additional incubation) treatments both showed suppressed emissions, and the free LF and the organo-mineral fractions had similar cumulative emissions throughout the experiment. This would suggest that the NaI had been effectively rinsed from the intra-aggregate LF, but perhaps not from the free LF or the organo-mineral fraction. SEM EDX carried out on isolated Sohi fractions from a forest soil indicated that residual NaI was greatest in the free LF and organo-mineral fractions (Burns, 2011). Thus it is not possible to rule out any influence of the NaI on respiration from the isolated SOM fractions.

Recombining any combination of isolated SOM fractions resulted in suppression of respiration compared to the summed equivalent fraction + sand treatments. Swanston *et al* (2002) observed a negative interaction when recombining light material and organo-mineral fractions and proposed that this could be due to reduced aeration in recombined treatments, the removal of natural soil structure by mixing SOM into a uniform matrix or microbial competition between species which are normally spatially separated in the soil matrix. However, respiration from the intra-aggregate LF + free LF + sand treatments were suppressed relative to the summed intra-aggregate LF + sand and free LF + sand treatments, suggesting that microbial competition is the more likely explanation in this instance.

These results suggest that the physical structure of the whole soil is crucial in stabilising SOC whilst maintaining nutrient cycling through microbial decomposition. In Chapter 4 FT-IR spectra of the intra-aggregate LF, free LF and organo-mineral fractions isolated from soil sampled from the same site (two years previously) indicated that the intra-aggregate LF was more decomposed than the free LF. The C:N ratios measured for the isolated free and intra-aggregate LF used in this experiment were lower than in the previous experiment, although this is likely due to seasonal variation in SOM quality (Trasar-Cepeda *et al*, 1998) as soil sampling took place in the spring of 2012 and autumn of 2014. The intra-aggregate LF had a much lower ^{14}C enrichment value and if we can assume that little

has changed in the two years between these sampling events, then this shows that older SOM is not necessarily recalcitrant and decomposition is being controlled by access to SOM by microbes, and not chemical recalcitrance.

6.4.2 Temperature sensitivity of SOC fractions

The response of different treatments to temperature sensitivity was surprisingly flat, with very little difference between samples or between temperature changes. A similar experiment incubating whole soil and isolated Sohi fractions from a forest soil found that all fractions were significantly more sensitive than the whole soil ($Q_{10} \sim 1.8$) and had significantly different Q_{10} values from each other with the free LF being the most sensitive ($Q_{10} \sim 3.0$) (Burns, 2011).

Fang *et al* (2005) incubated soil samples from different soil horizons (0–10 cm and 20–30 cm) to represent labile and recalcitrant SOM pools. They found that although temperature had an effect on the rate of respiration of each sample, there was no significant difference in the temperature sensitivity. Isotopic studies on arable soil (Conen *et al*, 2006) showed that temperature change had no effect on the age of C being respired.

Plante *et al* (2009) conducted three experiments (incubations of crushed or sieved whole soils, intact or crushed macro-aggregates, and recombined macro-aggregates) designed to test if physical protection increased temperature sensitivity of SOM decomposition. Their results showed no significant differences between any treatments, although they speculated that the crushing treatment may not have been sufficient to release all of the protected SOM.

Controlled laboratory incubations of soils are necessary for quantifying heterotrophic respiration in contrasting SOM fractions. Several researchers have discussed the limitations of calculating temperature sensitivity of SOM from laboratory incubations. A particular problem is that recalcitrant SOM is decomposed at a greater rate at higher temperatures (Cooper *et al*, 2011). The different rates of change of SOM quality at any given time point can lead to an underestimate of Q_{10} when calculated from C respired over a set incubation time, rather than the time taken for a set volume of C to be respired (Reichstein *et al*, 2000; Dalias *et al*, 2001). This was not deemed to be a relevant issue in this study as the Q_{10} values of the s15 and s25 sample sets for the temperature change from 25 to 35 °C converged over time and similar proportions of C were mineralised at each temperature in each sample set.

6.5 Conclusions

Respiration rates from the isolated intra-aggregate LF were higher than free LF highlighting the importance of the physical structure of the whole soil in stabilising C. This clearly indicates that there is a labile component of SOC which is protected from decomposition through physical stabilisation in the soil matrix. This labile component may be highly vulnerable to soil disturbance through management practices such as tillage. However, when incorporated in whole soil the intra-aggregate LF had no significant impact on CO₂-C emissions and so, contradictorily may not be an important rate modifier when considering the impacts of land use change and management on C emissions from soil.

The findings from the Q₁₀ calculations did not support the C quality theory and indicated that SOM respiration from whole soil and isolated fractions with different levels of physical and chemical protection are equally sensitive to temperature and that physical protection within aggregates did not reduce the temperature sensitivity of SOM. The median Q₁₀ values had an overall average of 1.1 and the average Q₁₀ value at the end of the three week incubations was 1.2. These values are below those generally assumed in single pool ecosystem models and for fast pools in multiple pool models, which would lead to an over estimate in the effect of temperature increase on soil respiration for this soil.

Overall our results provide direct evidence that physical protection of SOM in micro-aggregates controls soil C stability. Under changing global temperatures, improving the structure of vulnerable managed soils in a manner that assures the formation of stable soil aggregates could potentially protect or increase the size of the soil-stored C pool.

7 Conclusions and recommended future research

This research set out to investigate the impacts of common agricultural land-management practices on soil organic C (SOC) stability. The work described has made an original contribution to the knowledge of the vulnerability and resilience of SOC to loss under different land management practices, and also to the debate surrounding the relative importance of different SOC stabilisation mechanisms within the soil matrix.

7.1 Conclusions

Incubations of intact soils are an essential means of studying the impacts of land management practices on SOC stability through measurement of GHG emissions under controlled conditions. In Chapter 2, the stability of C (and the rate of N₂O emissions) in the grassland soils was most influenced by the availability of N and the soil texture. However, neither of these variables had a significant impact on the stability of C in the arable soils, indeed none of the measured variables explained a significant amount of the variation. However, the rate of C release from intact soil does not provide any information about the chemical and physical mechanisms which stabilise SOC. To investigate the mechanisms soil must be separated into pools with different SOC stabilisation mechanisms using soil fractionation techniques.

The stability of SOC was measured in terms of its physical location within the soil matrix (quantification of SOM fractions), its susceptibility to utilisation by microbes (respiration rates), and by chemical characterisation (C concentration, C:N, ¹⁴C contents and FT-IR analysis). The results from Chapter 4 show that estimating turnover of SOM using physical location within the soil alone is risky. Fractions which had high C:N and were expected to be labile (turnover of decades) were found to have a component of very old C (determined by ¹⁴C content). This result was reinforced by FT-IR analysis which showed a chemical composition consistent with the presence of a highly recalcitrant component, and HyPy analysis which confirmed the presence of black C in the soil.

An experimental comparison of the two commonly used Zimmermann *et al* (2007) and Sohi *et al* (2001) fractionation methods had not previously been undertaken. Although the fractions isolated from these two methods are not directly comparable as they are defined using different constraints (and therefore contain a different ‘mixture’ of soil components), it was possible to draw some parallels between the labile fractions. The Sohi free and intra-aggregate light fractions (LFs) were found to have distinctly different radiocarbon (¹⁴C) ages and as the Zimmermann POM contains the portion of these two fractions which are

> 63 μm in size (the remainder is allocated to the silt and clay fraction) it is somewhat comparable to the combined Sohi LFs. This suggests that to more fully satisfy the assumption that modellable SOM fractions have homogenous turnover rates, the inter- and intra-aggregate LFs should be considered separately.

The choice of fractionation method used for any particular study will, however, depend strongly on the purpose of the study. In Chapter 5 of this thesis it was deemed necessary to fully distinguish between different fractions of physico-chemically stabilised SOC (using the Zimmermann *et al* (2007) method) to gain a full understanding of the impact of land-use change on SOC stabilisation within the soil matrix. However, in Chapter 6 the main aim was to determine the reduced stability of intra-aggregate material after aggregate disruption by tillage and so further fractionation of physico-chemically stabilised fractions was not necessary.

The relative importance of physical and chemical protection has been debated extensively in the literature. The work in this thesis has provided a valuable and original addition to the discussion. In Chapter 5 the relative importance of physico-chemical stabilisation in highly stable micro-aggregates had reduced (lower proportion of total SOC) but despite an overall increase in SOC stocks the distribution of C among fractions had not otherwise changed significantly. However, C:N indicated that there had been a shift in preferential storage of fresh C in the highly stable aggregates to the chemically stabilised silt and clay fraction after a land-use change from arable to grassland. In Chapter 6 the addition of inter- and intra-aggregate SOM into intact soil (grassland soil which was in steady state) did not cause any increase in the CO_2 flux from the soil, in fact there was a slight suppression in some cases. This was in contrast to intra-aggregate material incubated in a sand matrix which had almost equal CO_2 emissions to the whole soil. This serves to highlight the importance of the soil matrix structure in stabilising vulnerable SOC.

Results from Chapter 5 showed that a change in land-use from tillage to grassland resulted in an increase in the relative SOC stock. It also confirmed the hypothesis that the labile fractions would be the most sensitive to land-use change. However, a surprising, but previously observed, result was the sensitivity of the residual SOC (rSOC) fraction (resistant to chemical oxidation) to land-use change; it was consistently more sensitive than the sand and stable aggregate fraction and more sensitive than the silt and clay fraction in all but one case. This brings into question the ability of fractionation methods to isolate, or perhaps even the existence of, a completely inert fraction of SOC. This study has shown that the rSOC fraction sampled from different treatment plots (applied for 23 years) within the same field

site displayed a wide range of sensitivity on conversion to grassland (22 years after the experiment was abandoned). However, in the RothC model, the rSOC fraction is allocated to the inert pool, leading to a discrepancy between the conceptual definition and the ecological properties of this fraction (discussed in Chapter 4). It may be appropriate to allocate the rSOC fraction to the inert pool when modelling SOC dynamics under steady state conditions but the results from Chapter 5 suggest that changes in the rSOC fraction need to be considered when modelling SOC dynamics for sites which have undergone land-use change until a new steady state is achieved.

Overall, the results from the experiments described in this thesis have highlighted the importance of improving or maintaining the soil structure for stabilising SOC. Disturbance of the soil matrix, particularly the disruption of aggregation, causes not only a loss of C but also a shift in the importance of different stabilisation mechanisms. Some interesting and unexpected results were observed which warrant further investigation.

7.2 Recommended future research

- Comparisons between turnover times of similar SOM fractions from two commonly used soil fractionation methods and a hot-water extraction method were hampered by the presence of black C in the soil used. It would be of great advantage to repeat this experiment with a range of soils which have been thoroughly screened for black C prior to ^{14}C dating. The results of such experiments would allow better comparisons to be made between studies which have used different fractionation methods, improve modelling of SOM dynamics and aid in the development of a standardised soil fractionation method suitable for isolating modellable SOM fractions with homogenous turnover rates.
- It would be worthwhile developing a fractionation procedure based on the Zimmermann *et al* (2007) method which discriminates between inter- and intra-aggregate light fractions. This would more fully satisfy the requirement that modellable SOM pools should have homogenous turnover rates. However, one of the strengths of the Zimmermann *et al* (2007) method is that it is a closed system (there should be 100% recovery of initial soil mass and SOC at the end of the fractionation procedure) and the introduction of an additional density separation step would risk losing a readily soluble portion of the SOC in the density separation medium and during the rinsing of the isolated fractions.
- As stated in the conclusion section above, relying on physical location within the soil alone as a measure of C stability is not advisable. Selected SOM fractions isolated from the South Road archived and 2014 soils (Chapter 5) are being analysed by FT-IR analysis to assess their chemical composition. Unfortunately this analysis was not completed at the time of thesis submission but will be included in any future publications resulting from this research.
- The results from Chapter 6 warrant further investigation into the absolute protection provided to SOM occluded in aggregates. Incubations using soils of different texture, from different climates and different land-uses would provide a strong insight into the importance of physical protection as a stabilisation mechanism.
- A chemically oxidised fraction could be isolated from the Sohi *et al* (2001) organo-mineral fraction by oxidising it in a similar manner to the silt and clay fraction in the Zimmermann *et al* (2007) method. This additional fraction could be included in any further incubation experiments following the design of the experiment in Chapter 6. This would allow an assessment of the level of

physico-chemical stabilisation by adsorption onto mineral surfaces and occlusion in highly stable micro-aggregates.

- Investigating correlations between isolated SOM fraction characteristics, C stability and bulk soil properties would allow the development of a ‘virtual fractionation’ database. This would, however, be a huge undertaking.

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Appendix 1: Daily greenhouse gas fluxes from incubated arable and grassland soils

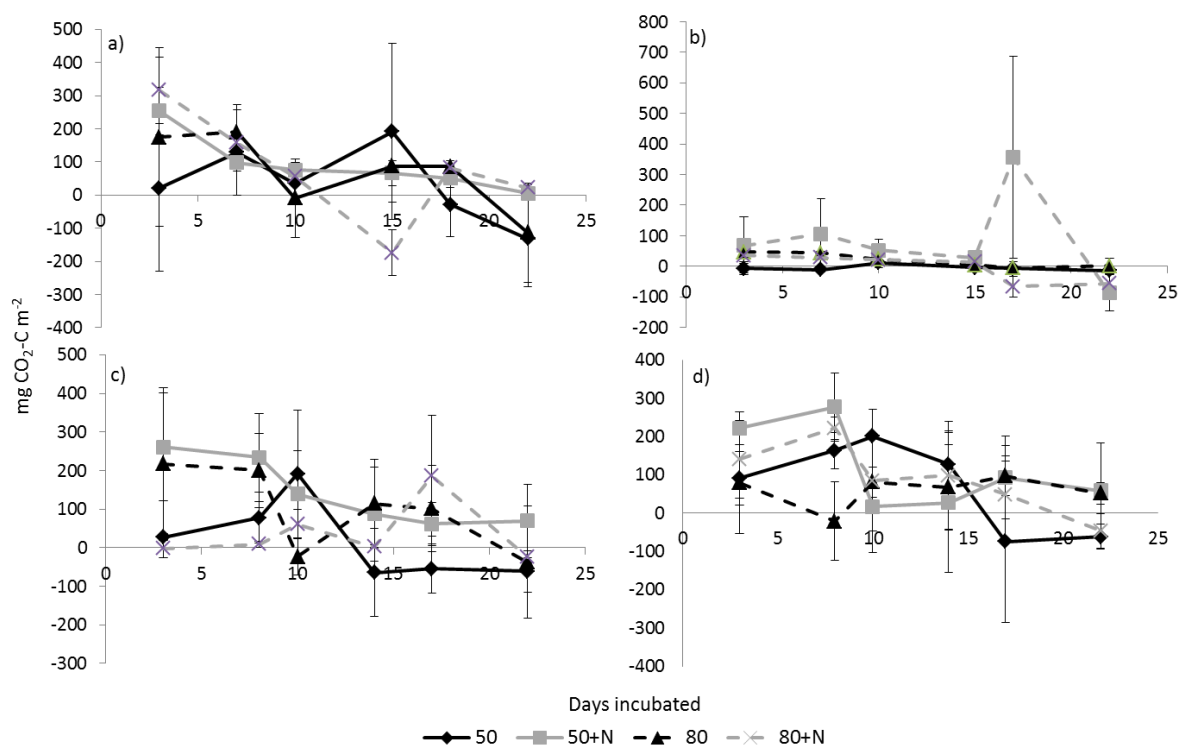


Figure A1.1: Daily $\text{CO}_2\text{-C}$ fluxes from four arable sites a) Gilchriston b) Woburn, c) Rosemaund and d) Boxworth. 50 – 50% water holding capacity (WHC), 50+N – 50% WHC with ammonium nitrate (AN) fertiliser, 80 – 80% WHC, 80+N – 80% WHC with AN fertiliser.

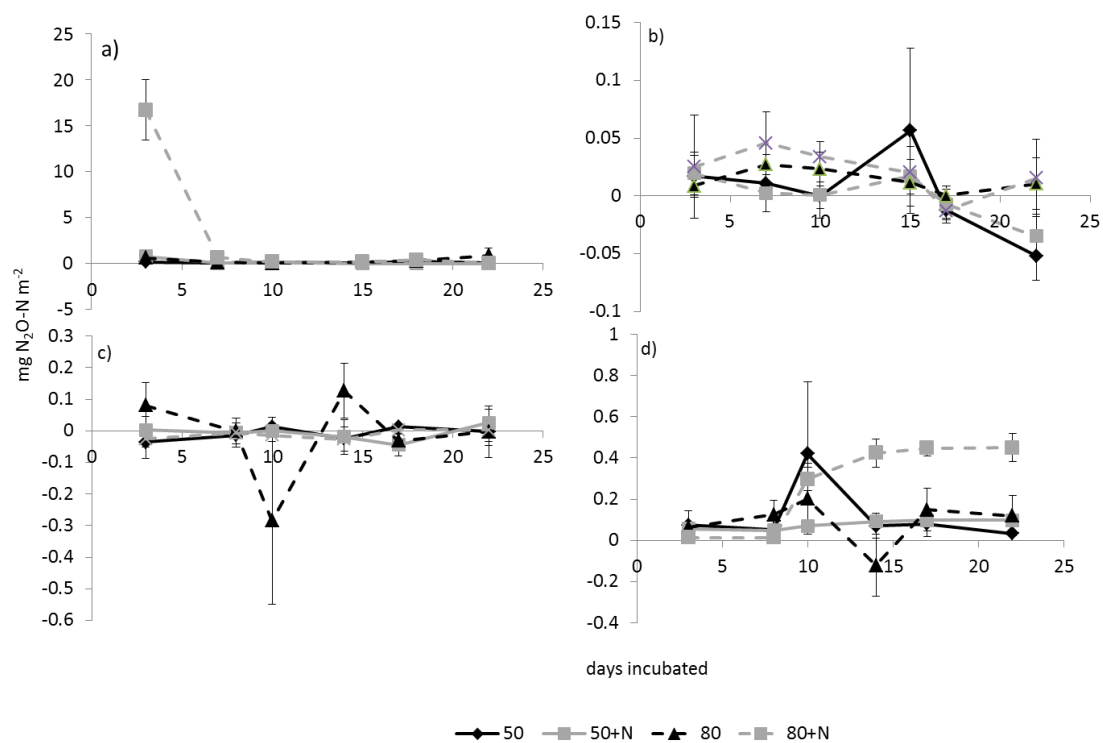


Figure A1.2: Daily $\text{N}_2\text{O-N}$ fluxes from four arable sites a) Gilchriston b) Woburn, c) Rosemaund and d) Boxworth. 50 – 50% water holding capacity (WHC), 50+N – 50% WHC with ammonium nitrate (AN) fertiliser, 80 – 80% WHC, 80+N – 80% WHC with AN fertiliser.

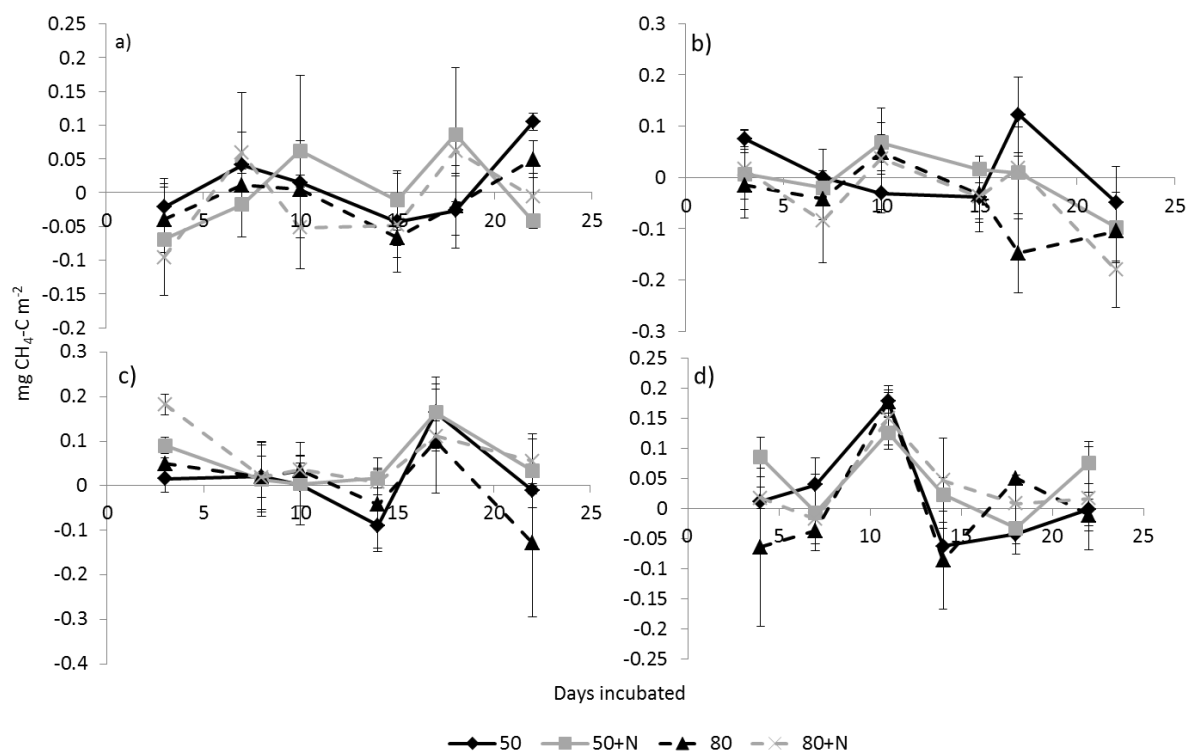


Figure A1.3: Daily $\text{CH}_4\text{-C}$ fluxes from four arable sites a) Gilchriston b) Woburn, c) Rosemaund and d) Boxworth. 50 – 50% water holding capacity (WHC), 50+N – 50% WHC with ammonium nitrate (AN) fertiliser, 80 – 80% WHC, 80+N – 80% WHC with AN fertiliser.

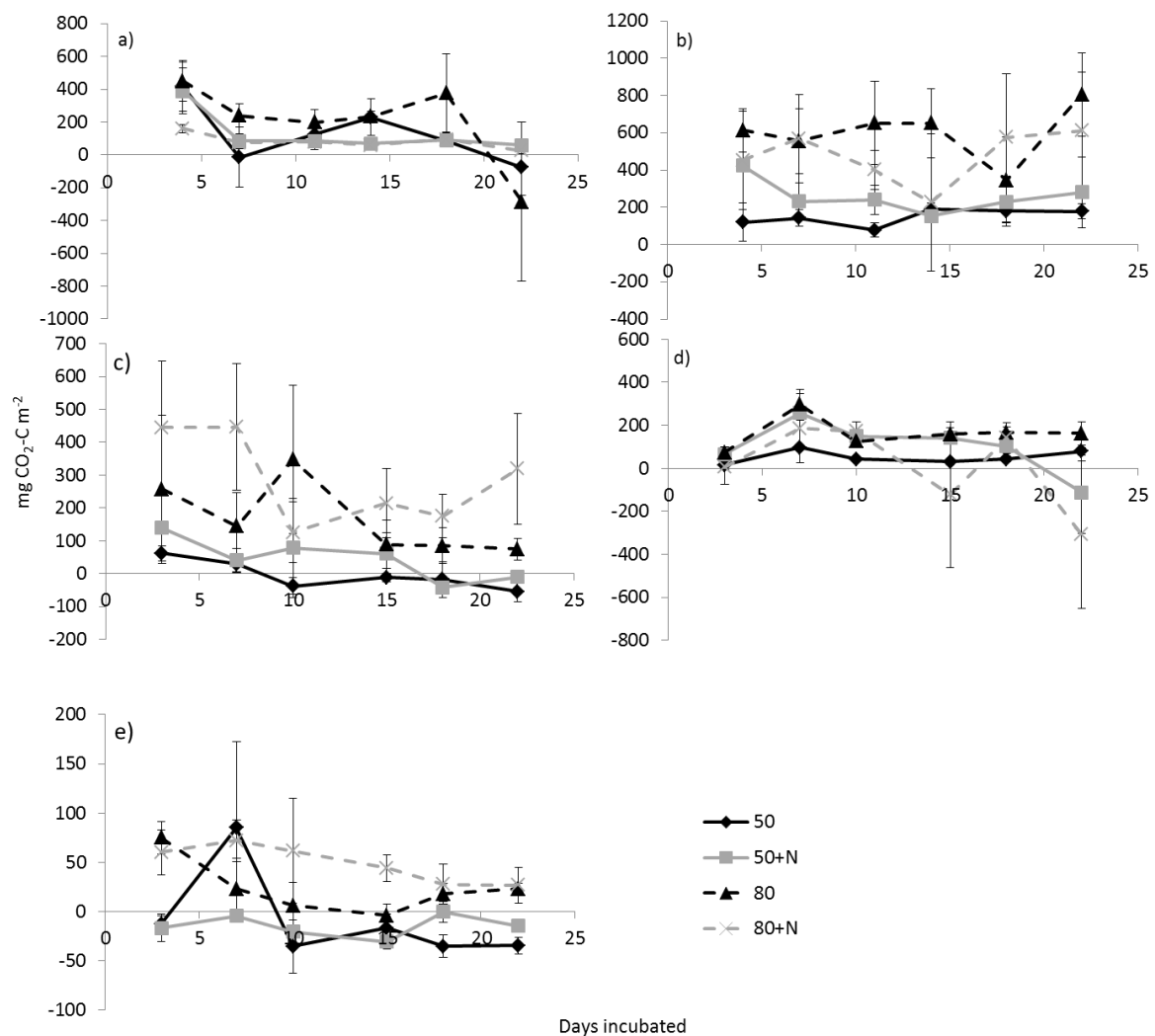


Figure A1.4: Daily $\text{CO}_2\text{-C}$ fluxes from five grassland sites a) Crichton b) Drayton, c) Hillsborough, d) North Wyke and e) Pwllpeiran. 50 – 50% water holding capacity (WHC), 50+N – 50% WHC with ammonium nitrate (AN) fertiliser, 80 – 80% WHC, 80+N – 80% WHC with AN fertiliser.

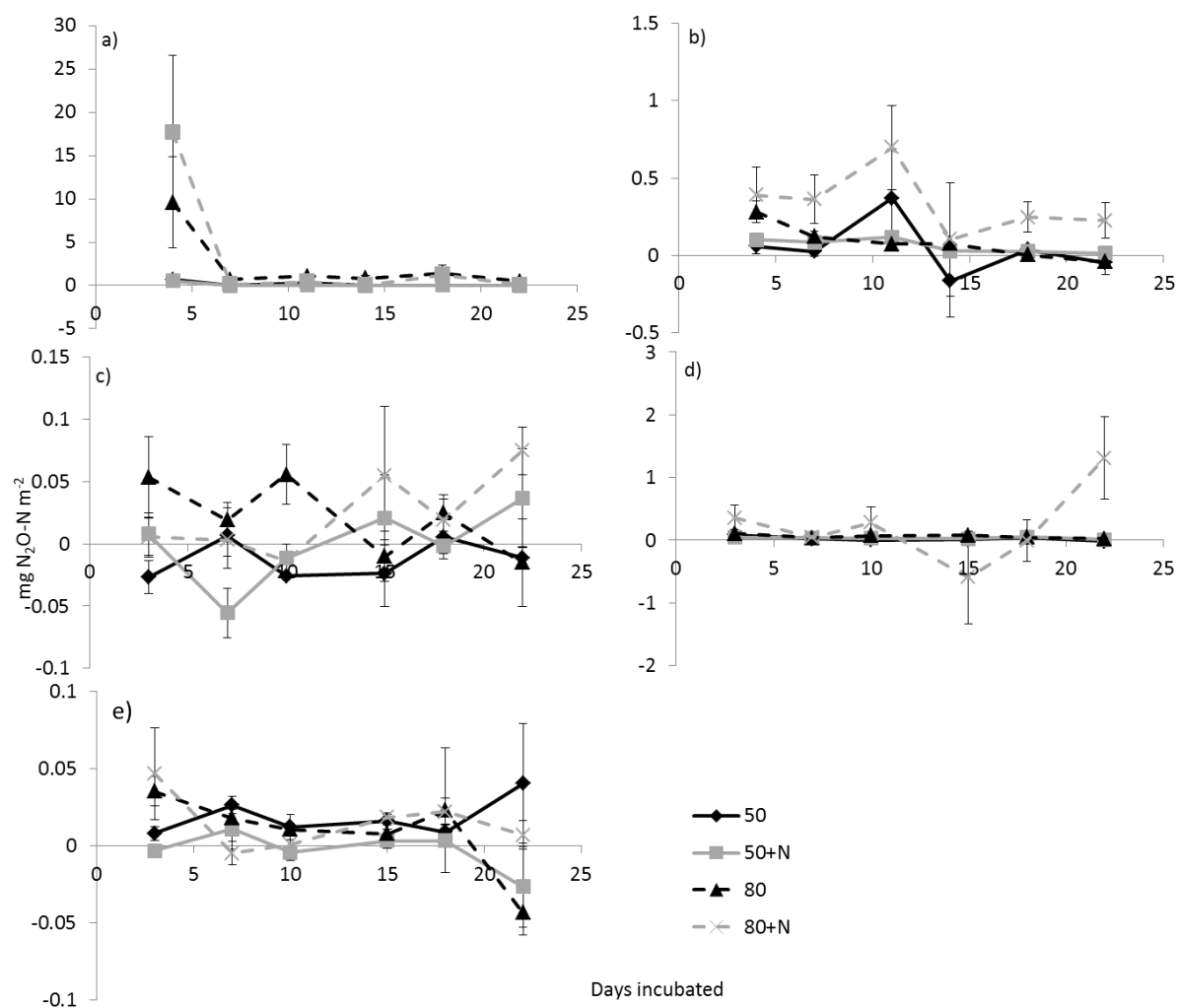


Figure A1.5: Daily $\text{N}_2\text{O-N}$ fluxes from five grassland sites a) Crichton b) Drayton, c) Hillsborough, d) North Wyke and e) Pwllpeiran. 50 – 50% water holding capacity (WHC), 50+N – 50% WHC with ammonium nitrate (AN) fertiliser, 80 – 80% WHC, 80+N – 80% WHC with AN fertiliser.

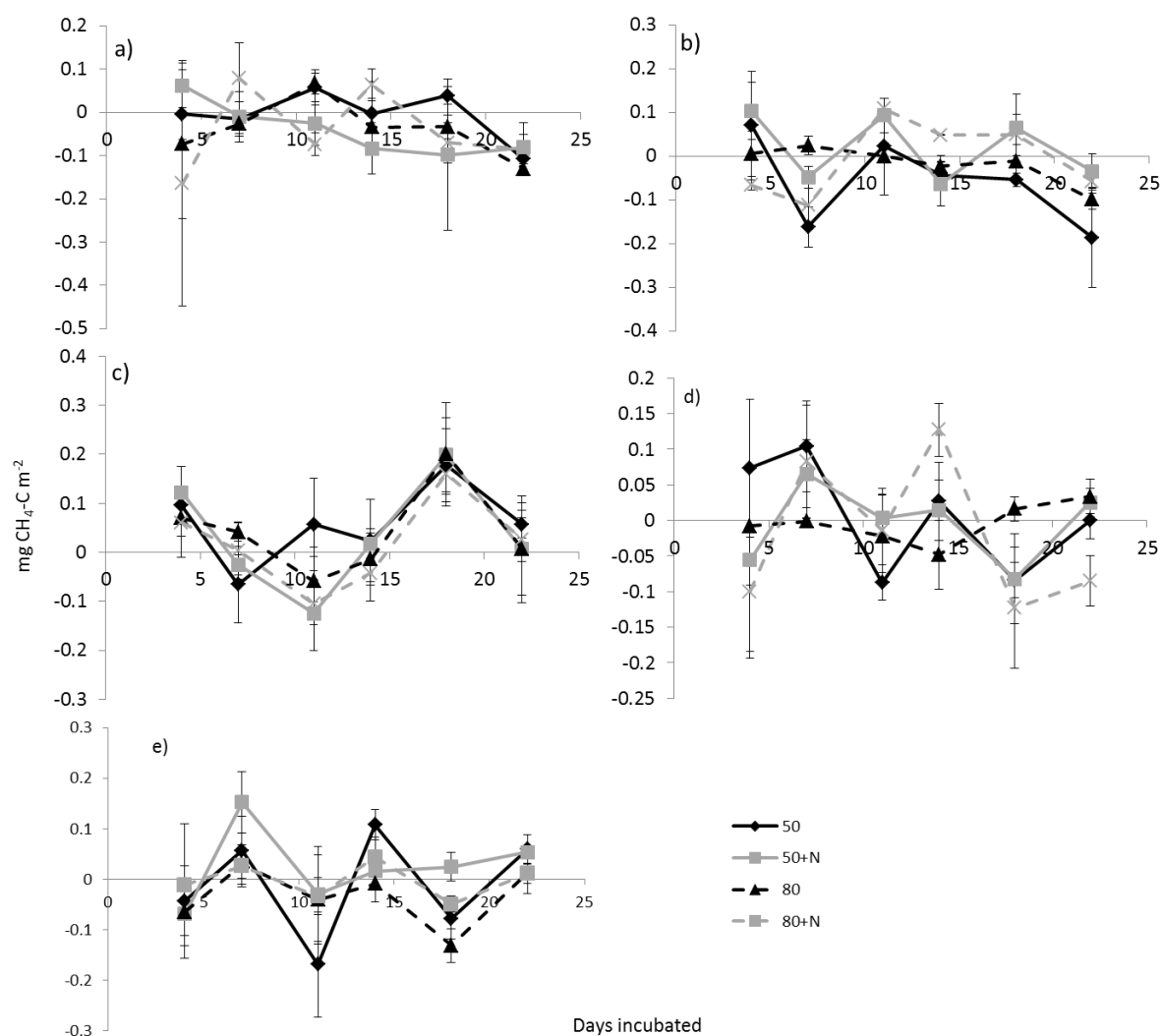


Figure A1.6: Daily $\text{CH}_4\text{-C}$ fluxes from five grassland sites a) Crichton b) Drayton, c) Hillsborough, d) North Wyke and e) Pwllpeiran. 50 – 50% water holding capacity (WHC), 50+N – 50% WHC with ammonium nitrate (AN) fertiliser, 80 – 80% WHC, 80+N – 80% WHC with AN fertiliser.

Appendix 2: Output from mean residence time modelling

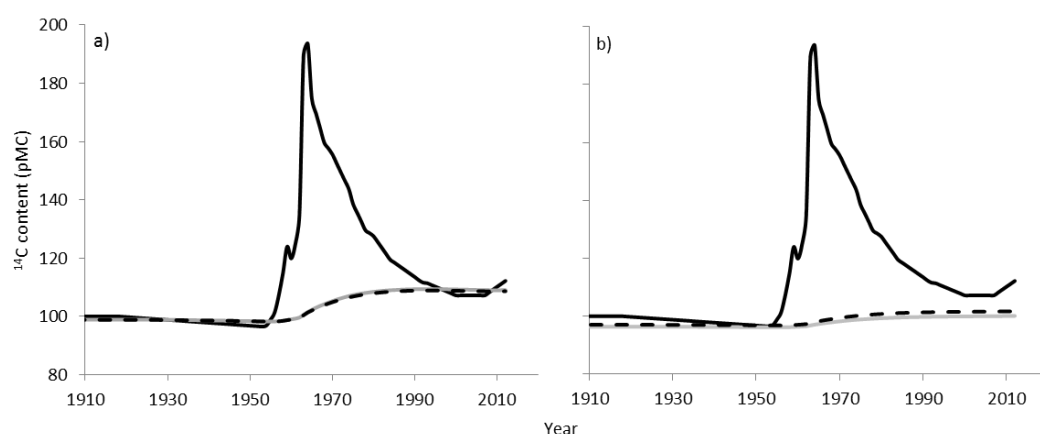


Figure A2.1: Bomb ^{14}C peak and modelled output for duplicate a) water soluble C and b) hot-water extractable C isolated using the method of Ghani *et al* (2003) expressed as percent modern C (pMC). The grey line represents duplicate 1 and the dashed black line represents duplicate 2.

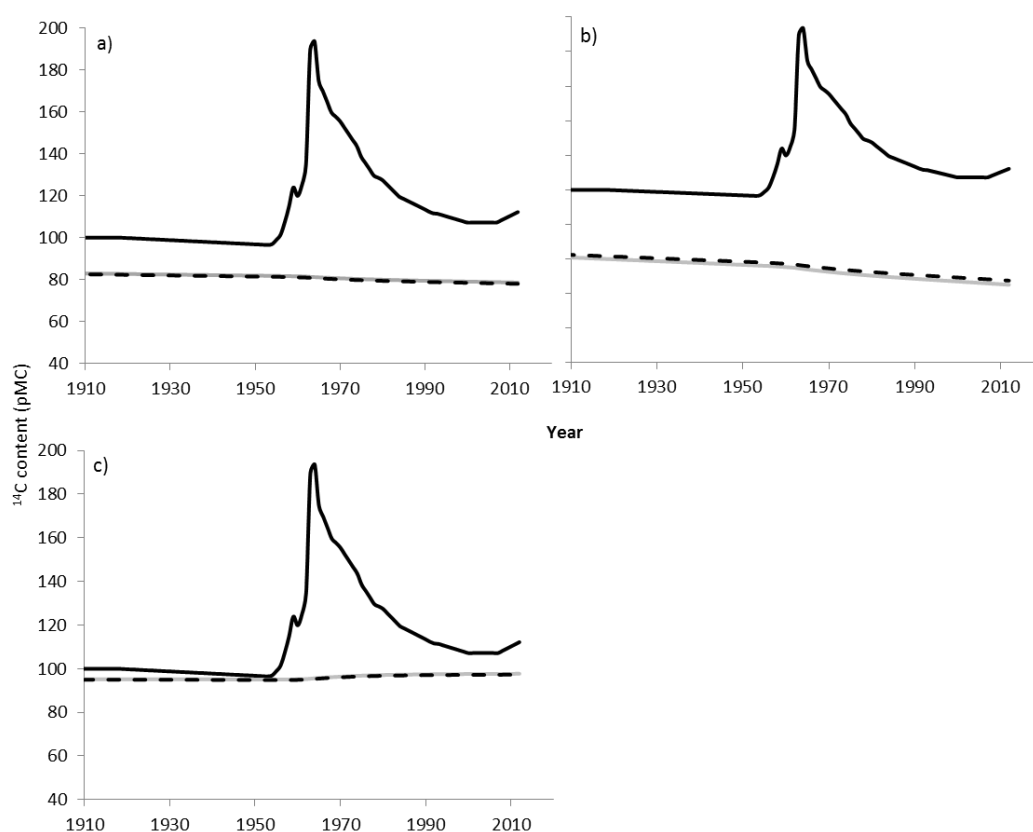


Figure A2.2: Bomb ^{14}C peak and modelled output for duplicate a) free light fractions (LF), b) intra-aggregate LF and c) organo-mineral fraction isolated using the method of Sohi *et al* (2001) expressed as percent modern C (pMC). The grey line represents duplicate 1 and the dashed black line represents duplicate 2.

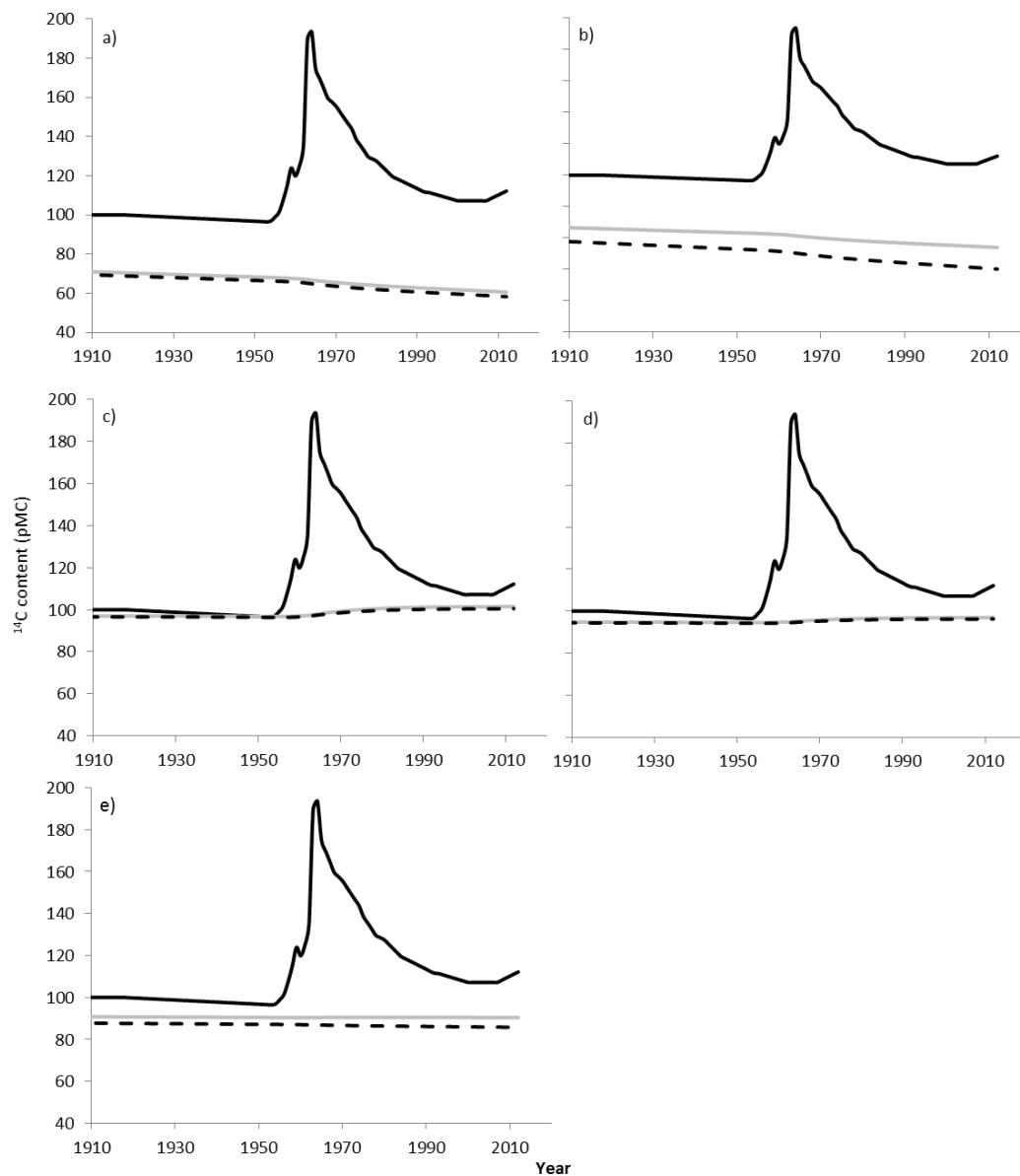


Figure A2.3: Bomb ^{14}C peak and modelled output for duplicate a) particulate organic matter, b) dissolved organic C, c) silt and clay, d) residual soil organic C and e) sand and aggregate fractions isolated using the method of Zimmermann *et al* (2007) expressed as percent modern C (pMC). The grey line represents duplicate 1 and the dashed black line represents duplicate 2.

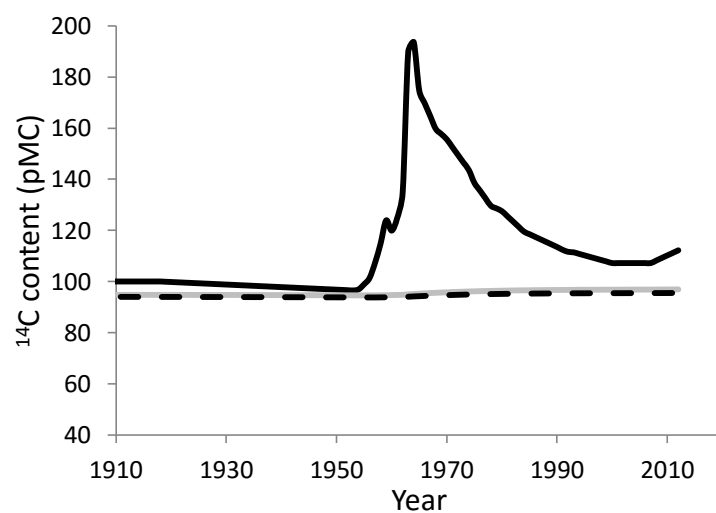


Figure A2.4: Bomb ^{14}C peak and modelled output for duplicate whole soil samples expressed as percent modern C (pMC). The grey line represents duplicate 1 and the dashed black line represents duplicate 2.

Appendix 3: Spectroscopic analyses of archived South Road soil extracts and SOC stocks by fraction.

Table A3.1: Specific UV absorbance at 254 nm ($SUVA_{254}$), in units of $L\ mg\ C^{-1}\ m^{-1}$, for water soluble C (WSC) and hot-water extractable C (HWE) from archived (1975) South Road tillage treatment plots at 6 cm intervals (isolated using the Ghani *et al* (2003) method (see Section 2.2.3 for methodology). Values for each extract within a depth followed by different letters are significantly different from each other (one-way ANOVA, $p < 0.05$).

Depth (cm)	Chisel Plough		Direct Drilled		Shallow Plough		Deep Plough	
	WSC	HWE	WSC	HWE	WSC	HWE	WSC	HWE
0–6	3.53b	3.79c	1.89a	2.37b	2.09a	2.45b	2.23ab	1.87a
6–12	3.29b	3.75b	2.29ab	2.42a	1.88a	2.50a	-	-
12–18	3.42b	3.80b	1.83a	2.54a	1.71a	2.65a	-	-
18–24	3.86c	3.81d	2.17b	2.33b	1.70a	2.75c	2.20b	1.79a
24–30	3.67c	4.40c	2.37ab	2.72b	1.79a	2.55b	2.50b	1.82a
30–36	4.50c	4.87c	1.72a	2.72b	-	-	2.28b	1.29a

Table A3.2: E_4E_6 ratios for water soluble C (WSC) and hot-water extractable C (HWE) from archived (1975) South Road tillage treatment plots at 6 cm intervals (isolated using the Ghani *et al* (2003) method (see Section 2.2.3 for methodology). Values for each extract within a depth followed by different letters are significantly different from each other (one-way ANOVA, $p < 0.05$).

Depth (cm)	Chisel Plough		Direct Drilled		Shallow Plough		Deep Plough	
	WSC	HWE	WSC	HWE	WSC	HWE	WSC	HWE
0–6	3.10	3.26a	7.07	2.89a	6.61	13.65ab	4.42	18.30b
6–12	1.45	4.24	3.65	3.45	6.68	4.60	-	-
12–18	3.38	3.17	6.12	6.31	4.91	8.20	-	-
18–24	3.14	6.41	6.51	2.80	8.36	7.25	5.78	10.95
24–30	4.67	8.29ab	5.35	2.95a	7.60	6.72ab	3.79	23.57b
30–36	2.71	5.82a	5.62	5.63a	-	-	6.11	33.28b

Table A3.3: Absolute mean soil organic C (SOC) stocks of soil organic matter fractions isolated using the Zimmerman *et al* (2007) fractionation method (see Section 2.2.2 for methodology) from 1975 and 2014 South Road soil at three depths. Standard errors are shown in parenthesis. S+A – sand and aggregates, s+c – silt and clay, rSOC – residual SOC, POM – particulate organic matter, DOC – dissolved OC.

		SOC stocks by fraction (Mg C ha ⁻¹)									
Treatment	Depth (cm)	S+A		s+c - rSOC		POM		DOC		rSOC	
		1975	2014	1975	2014	1975	2014	1975	2014	1975	2014
Chisel Plough	0–6	1.90 (0.04)	1.02 (0.27)	9.23 (0.49)	12.14 (0.85)	6.41 (0.11)	4.81 (0.45)	0.77 (0.05)	0.37 (0.02)	11.48 (0.42)	7.91 (0.40)
	18–24	3.16 (0.07)	1.64 (0.34)	11.41 (1.39)	12.39 (1.78)	4.54 (0.20)	9.38 (2.88)	0.65 (0.04)	0.87 (0.14)	8.72 (0.99)	10.58 (1.44)
	30–36	0.97 (0.11)	0.84 (0.27)	5.62 (0.72)	5.90 (1.32)	1.26 (0.11)	5.01 (0.79)	0.38 (0.08)	0.71 (0.02)	4.62 (1.14)	6.50 (1.78)
Direct Drill	0–6	2.07 (0.10)	1.94 (0.37)	6.78 (0.92)	10.64 (1.64)	9.81 (0.57)	5.23 (0.60)	1.06 (0.16)	0.74 (0.11)	15.80 (1.78)	11.93 (1.57)
	18–24	2.86 (0.36)	1.40 (0.25)	10.76 (0.99)	14.34 (1.10)	2.98 (0.37)	9.94 (1.61)	0.30 (0.02)	0.88 (0.13)	4.60 (0.21)	8.52 (1.30)
	30–36	-	1.43 (0.06)	-	5.54 (0.68)	-	3.67 (0.98)	-	0.58 (0.04)	-	5.21 (0.40)
Deep Plough	0–6	1.93 (0.15)	1.23 (0.31)	8.51 (0.98)	12.96 (1.74)	4.96 (1.15)	7.37 (1.56)	0.50 (0.04)	0.59 (0.09)	8.87 (0.81)	10.05 (1.13)
	18–24	2.48 (0.34)	1.03 (0.07)	10.87 (0.15)	13.02 (0.57)	4.72 (0.45)	6.22 (0.46)	0.49 (0.13)	0.68 (0.10)	8.27 (0.47)	9.09 (1.00)
	30–36	2.21 (0.52)	0.49 (0.17)	5.74 (1.84)	6.55 (1.44)	4.85 (0.15)	2.76 (0.59)	0.60 (0.06)	0.33 (0.04)	8.18 (0.72)	5.49 (0.64)
Shallow Plough	0–6	2.79 (0.45)	1.03 (0.19)	9.56 (1.50)	15.20 (1.07)	7.87 (0.64)	4.54 (0.58)	0.73 (0.04)	0.66 (0.04)	10.91 (1.30)	9.21 (0.31)
	18–24	4.05 (0.35)	1.82 (0.33)	14.77 (0.85)	14.95 (0.83)	4.26 (0.49)	6.74 (0.90)	0.52 (0.09)	0.66 (0.07)	7.34 (0.56)	6.70 (0.33)
	30–36	2.23 (0.36)	0.53 (0.26)	4.40 (0.10)	8.55 (0.20)	9.95 (1.24)	0.99 (0.38)	1.28 (0.12)	0.30 (0.02)	19.08 (0.18)	6.51 (0.09)

Appendix 4: Average CO₂ –C fluxes by sampling day from incubated fraction and whole soil combinations.

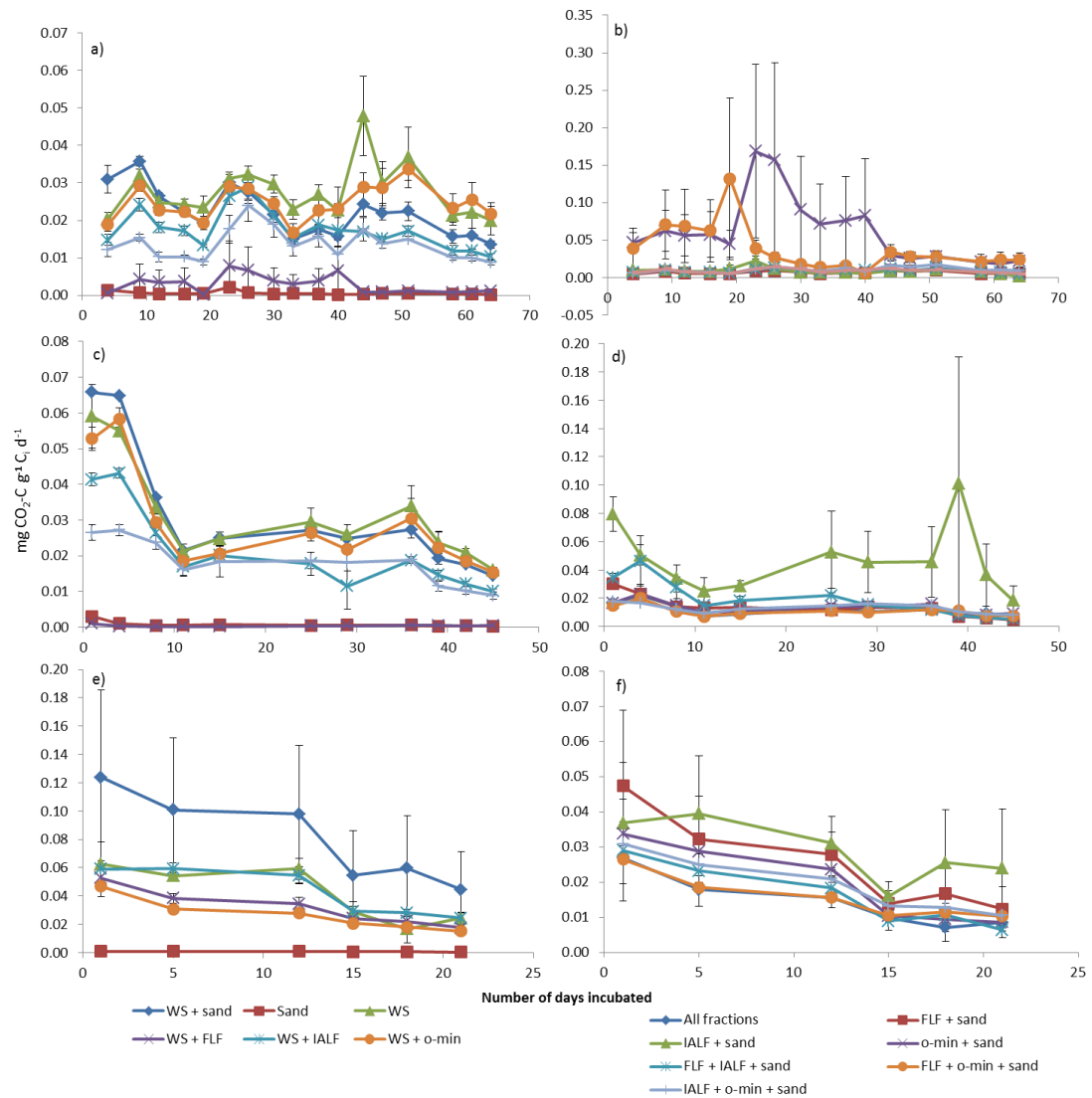


Figure A4.1: Average CO₂-C emissions (mg CO₂-C g⁻¹ C_i d⁻¹) from a) s15 control, whole soil + fraction samples and b) reconstituted soil combinations sequentially incubated from 15 °C (21 days at each of 15 (n=9), 25 (n=6) and 35 °C (n=3)), c) s25 control, whole soil + fraction samples and d) reconstituted soil combinations sequentially incubated from 25 °C (21 days at each of 25 and 35 °C (n=3)), e) s35 control, whole soil + fraction samples and f) reconstituted soil combinations sequentially incubated at 35 °C for 21 days (n=3). WS – whole soil, FLF – free light fraction, IALF – intra-aggregate LF, o-min – organo-mineral fraction.

Appendix 5: Organic inputs to grassland and arable soils

A5.1 Introduction

Two sites in Scotland, one arable and one grassland, were part of an intensive study investigating the impacts of organic inputs (in the form of slurry, farm yard manure and poultry litter on the arable site and dung and urine on the grassland site) on GHG emissions from the soil (Bell *et al*, 2016; Bell *et al*, 2015a). An additional objective considered the effect of adding biochar to slurry and applying both to soil (Winning, 2014). To further utilise these experiments, the impacts on the soil organic C (SOC) stocks were investigated.

A5.2 Materials and Methods

A5.2.1 Boghall organic fertiliser experiment - site, treatment structure and analyses

Site

Boghall is a commercial and research farm to the south of Edinburgh on SRUC's Bush estate (grid reference NT 248654). The field to which treatments were applied was a sandy loam arable soil belonging to the 'Darvel' soil series which had been sown to spring barley for the previous four years.

Treatment structure and soil sampling

The experimental design of the field trial is described in detail in Bell *et al* (2016) and Winning (2014). The analysis of SOC stocks presented here took account of the control, surface broadcast slurry, slurry + biochar, layer manure and poultry litter treatments applied in the spring of 2013 only. Briefly, treatments were applied to 6 x 12 m plots in a randomized block design (n = 3). The biochar was produced from oil seed rape straw pellets, at a pyrolysis temperature of 550 °C, at the UK Biochar Research Centre. Prior to application on the plots, the biochar was mixed into tanks with the slurry, at a mass ratio of 1:2 slurry dry matter, and stored for 50 days before application. Slurry was applied to the plots at a rate of 41.7 t ha⁻¹ (Bell *et al*, 2016). Selected properties for slurry and biochar are given in Table A5.1.

All plots were sown with winter wheat in October 2013 by direct drilling and herbicide and pesticide were applied in line with standard recommendations. Soil was sampled from the plots one, six and twelve months after treatment application. One bulk density core was extracted at random from each plot and five samples were taken randomly across each plot to

a depth of 10 cm using an auger. Soil samples were stored in a freezer at -5 °C in sealed plastic bags until analysis was carried out (within 3 months).

Table A5.1: Selected slurry properties (Winning, 2014; Bell *et al*, 2016)

	Slurry properties	Biochar properties
pH	6.89	8.44
Dry matter (%)	1.6	-
C applied (t C ha ⁻¹)	21.95	14.1
C:N	4.79	226
Moisture (%)	-	4.12
Ash content (%)	-	18.30

Analyses

Triplicate air dried and ball milled sub-samples from all plots were analysed for C and N concentrations using a Flash elemental analyser and used to calculate average SOC stocks for each treatment using the measured bulk densities for each plot.

Microbial biomass was determined for the slurry, slurry + biochar and control treatment plots only. Triplicate sub-sample microbial biomass C (MBC) was determined using the chloroform-fumigation-extraction method. Soil (10 g) was weighed into either a 120 ml glass jar or a 250 ml plastic pot (three of each). The samples in glass jars were placed in a vacuum oven on selves lined with dampened tissue paper along with a beaker containing 30 ml of chloroform (with a few anti-bump granules) and a beaker containing approximately 25 ml of soda lime. The oven was then evacuated until the chloroform was seen to boil vigorously and sealed for 24 hours.

After the 24 hour fumigation, the tissue, chloroform and soda lime were removed from the oven. The oven was then repeatedly evacuated into a fume cupboard until there was no detectable smell of chloroform from the soil samples.

At the beginning of the fumigation period, the samples in the plastic pots were extracted as follows: 0.5 M K₂SO₄ was added to the soil at a ratio of 4 ml to 1 g soil, the pots were placed on an orbital shaker for 30 minutes at 120 rpm. After shaking, the solution was decanted into a 50 ml centrifuge tube and centrifuged for 15 minutes at 5000 rpm. The supernatant was then transferred into a plastic scintillation vial and placed in a fridge at 5 °C. Fumigated samples were extracted in the same way at the end of the fumigation period.

Extracts were analysed for dissolved organic C (DOC) using a Rosemount-Dohrmann DC-80 TOC analyser. Samples were diluted 1:1 with a 5% Sodium Hexameta-phosphate solution (adjusted to pH 2.1 using orthophosphoric acid) before analysis.

Microbial biomass C (MBC) was then calculated using the following equation:

$$\text{MBC} = \frac{\text{Fumigated DOC} - \text{Unfumigated DOC}}{0.45}$$

Whole soil sub-samples from the control, slurry and slurry + biochar plots were chemically characterised by FT-IR spectroscopy. See section 3.2.3 for methodology, peak assignments were made according to Palacio *et al* (2014).

A5.2.2 Crichton dung and urine application experiment - site, treatment structure and analyses

Treatment structure and soil sampling

The Crichton site has been described in detail in Chapter 5 and the experimental design has been described in detail by Bell *et al* (2015b) (this analysis of SOC stocks does not take the plots treated with DCD in to consideration). Briefly, in the spring of 2012 treatments were applied to 6 x 2 m plots in a randomised block design. The treatments were dung (D), urine (U), synthetic urine (SU) and a no treatment control (C). Soil samples were collected in the same manner as at the Boghall site in spring 2012, autumn 2012 and spring 2013. Samples were sieved to < 4 mm, air dried and stored in sealed plastic bags. One bulk density core was taken from each plot in spring and autumn of 2013.

Analyses

Concentrations of C and N and SOC stocks were determined as described for the Boghall site.

A5.2.3 Statistical analyses

Repeated measures ANOVA were used to analyse MBC, SOC stocks and C:N. All statistical analyses were carried out in Genstat (v15.1).

A5.3 Results

A5.3.1 Boghall microbial biomass carbon

The repeated measures ANOVA showed that there were no significant differences in MBC between treatments, however there was a slight effect of time ($p = 0.051$).

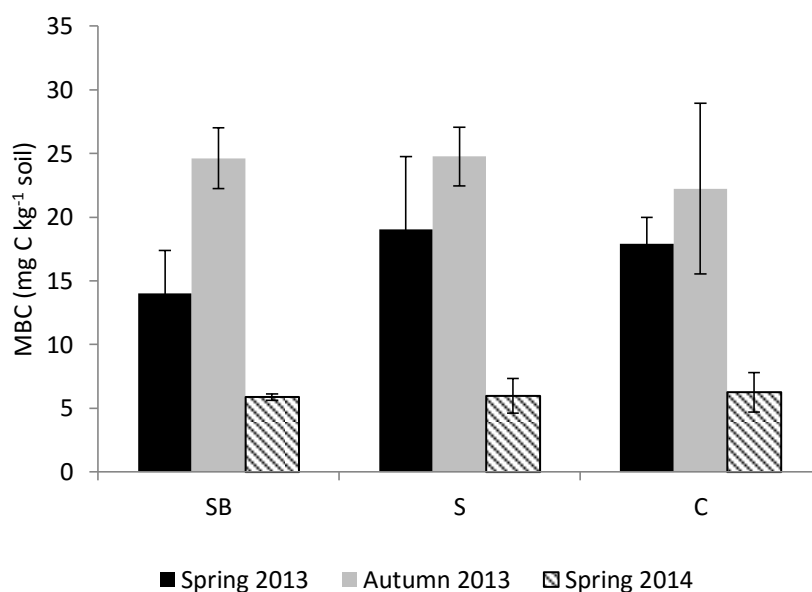


Figure A5.1: Microbial biomass C (MBC) for slurry + biochar (SB), slurry (S) and control (C) plots at three time points, spring 2013, autumn, 2013 and spring 2014 (1, 6 and 12 months after treatment application, respectively).

A5.3.2 Boghall SOC stocks and C:N ratios

The slurry treatment had significantly lower C stocks than all other treatments ($p < 0.001$) one month after treatment application; however this was no longer apparent at 12 months after application (Table A5.2). There were no significant differences between C:N (Table A5.2).

Table A5.2: Soil organic C (SOC) stocks and C:N for soil sampled in spring 2013 (one month after treatment application) and spring 2014 (12 months after treatment application). Standard errors are shown in parenthesis.

Treatment	Year	C stock (t C ha ⁻¹)	C:N
control	2013	23.86 (1.45)	19.13 (1.50)
	2014	24.47 (1.45)	16.48 (3.42)
slurry	2013	21.24 (1.37)	19.72 (0.64)
	2014	22.58 (1.43)	16.27 (2.45)
slurry + biochar	2013	24.55 (1.46)	19.32 (0.64)
	2014	24.45 (1.73)	18.60 (0.56)
Layer manure	2013	24.57 (1.00)	17.99 (0.91)
	2014	24.60 (1.40)	21.84 (8.17))
Poultry litter	2013	23.62 (1.81)	18.38 (1.08)
	2014	24.49 (2.24)	19.36 (1.64)

A5.3.2 Boghall FT-IR analysis

No notable differences in the chemical structure of the whole soils from the control, slurry or slurry + biochar plots were detected (Figure A5.2).

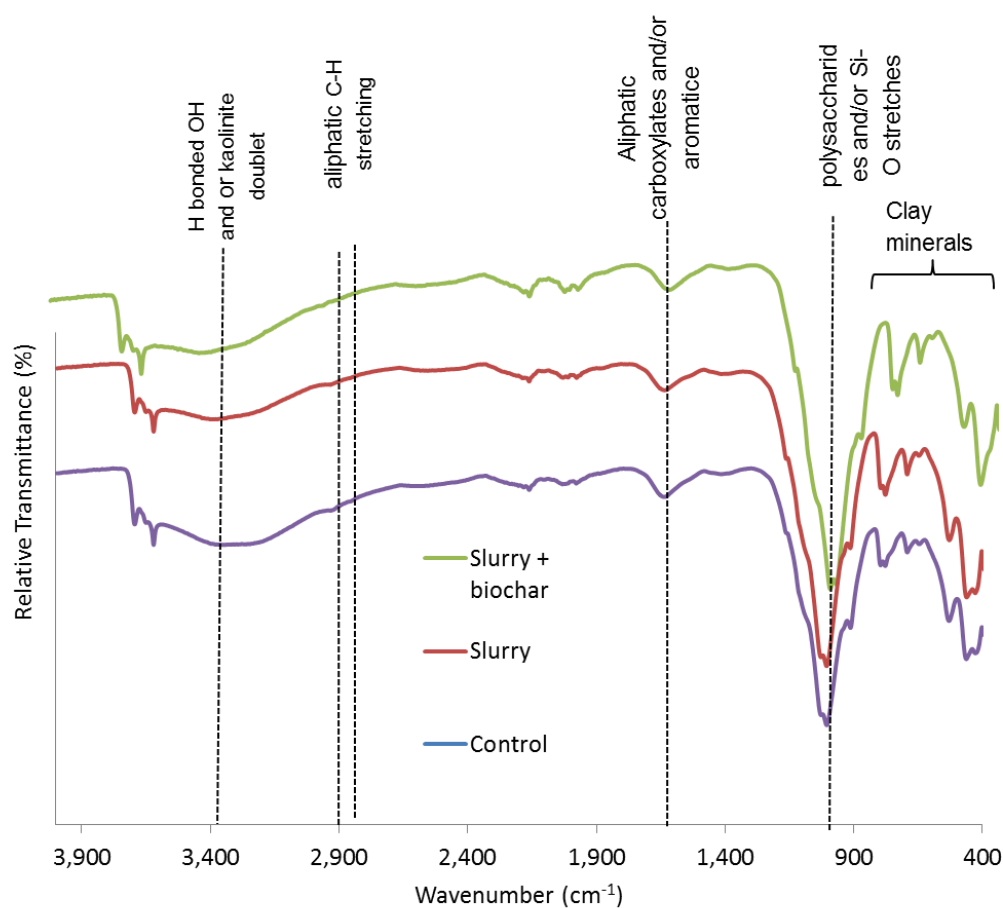


Figure A5.2: FT-IR spectra for the Boghall control, slurry and slurry + biochar treatment whole soils.

A5.3.3 Crichton SOC stocks and C:N

There were no significant differences in SOC stocks or C:N between treatments and there was no effect of time.

Table A5.3: Soil organic C (SOC) stocks and C:N ratios for soil sampled in spring 2012, spring 2013 and autumn 2013 for synthetic urine, urine, dung and control treatments. Standard errors are shown in parenthesis where they were ≥ 0.01 .

Treatment	Time	SOC stocks (t C ha ⁻¹)	C:N
Synthetic Urine	Spring 2012	21.03	10.70
	Spring 2013	22.58	11.08 (0.08)
	Autumn 2013	21.99	11.22 (1.00)
Urine	Spring 2012	24.53	10.74 (0.24)
	Spring 2013	26.84	10.80 (0.22)
	Autumn 2013	26.43	10.69 (0.71)
Dung	Spring 2012	23.18	10.95 (0.19)
	Spring 2013	20.48	10.79 (0.23)
	Autumn 2013	20.03	10.17 (0.58)
Control	Spring 2012	27.43	10.80 (0.26)
	Spring 2013	26.54	10.78 (0.24)
	Autumn 2013	25.45	10.21 (1.06)

A5.4 Discussion and Conclusions

Organic amendments have been shown to increase both microbial biomass and SOC stocks in agricultural soils (Bhattacharyya *et al*, 2010; Liang *et al*, 2012; Yu *et al*, 2012; Powlson *et al*, 2012). Biochar has also been shown to increase SOC and microbial biomass (Liu *et al*, 2015). Dung C (8% originally applied) has been identified in grassland soil one year after application (Dungait *et al*, 2005). Urine has been shown to have a priming effect leading to a net loss of SOC (Hamer *et al*, 2009).

However, Bittman *et al* (2005) found that multi-year application of cattle slurry to grassland increased the biomass of bacteria relative to untreated plots, but reduced the biomass of fungi resulting in there being no significant difference in MBC between manured and control treatments, this phenomenon persisted for two years after the application of manure ceased. Bol *et al* (2003) also found no significant difference in MBC four weeks after slurry application to grassland.

The IPCC (2006a) recommends a net SOC increase factor of 1.37 for temperate soils with high rates of manure inputs over a 20 year period. Taking this into consideration, it was concluded that a single organic fertilisation event was not sufficient to cause any significant change in SOC stocks.

